SOIL PHOSPHORUS DYNAMICS UNDER ANNUAL OR SINGLE APPLICATIONS OF ALKALINE TREATED BIOSOLIDS

by

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Abstract

Land application of biosolids as a soil fertility amendment is an alternative to traditional disposal methods. The influence of annual Alkaline Treated Biosolids (ATB) land application on phosphorus (P) dynamics in acidic soils needs to be better understood for more efficient P management. This study examines plant uptake, plant available, soil total phosphorus (P) content and potential P accumulation as a function of rate and frequency of application of an ATB amended to an agricultural soil over four years. The results suggest that applying high rates of ATB annually can increase the soil pH, Mehlich 3 and water extractable P, and crop P uptake in an acidic agricultural soil. No significant soil P accumulation was observed and P related environmental concerns were limited in this study. Potential soil P accumulation and higher P loss needs to be considered with annual high ATB rates application over a longer period study.

List of Abbreviations and Symbols Used

AA	Alkaline admixture
AASSAD	Advanced Alkaline Stabilization with Subsequent Accelerated Drying
Al	Aluminum
ATB	Alkaline treated biosolids
BPU	Biomass phosphorus uptake
C	Carbon
CaO	Lime
CKD	Cement kiln dust
Fe	Iron
GPU	Grain phosphorus uptake
ICP	Inductively coupled plasma
ICP-OES	Inductively coupled plasma optical emission spectrometry
LKD	Lime kiln dust
M3Al	Mehlich 3 extractable aluminum
M3Fe	Mehlich 3 extractable iron
M3P	Mehlich 3 extractable phosphorus
N	Nitrogen
P	Phosphorus
PSI	Phosphorus saturation index
SOM	Soil organic matter
STP	Soil test P
Soil TP	Soil total phosphorus
WEP	Water extractable phosphorus

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1 Introduction

1.1 Overview

The National Sewage Report Card identified that over three trillion liters of wastewater are generated in Canada each year (Sierra Legal Defence Fund. 2004) and the quantity is expected to increase. Although sewage treatment plants have been built in most municipalities across Canada, large amounts of raw sewage are still being discharged into freshwater bodies. The quality of the treated wastewaters differs due to variability in the quality standards applied within each provincial jurisdiction (Chambers et al. 1997). In Nova Scotia, the term "biosolids" is used to describe the secondary treated sewage where pathogens and heavy metal contents have been significantly reduced (Nova Scotia Department of Environment 2010).

Biosolids are high in organic matter content, nitrogen (N) and phosphorus (P), and have low carbon (C)/N ratios which make them viable as soil amendments and nutrient sources to enhance soil physical properties and plant yield (Adani and Tambone 2005; Kinney et al. 2006). There are several methods for biosolids disposal but none of them is risk free. One of the prevalent biosolids disposal methods is land application. The latest report states that more than 660,000 dry tonnes of biosolids and sludge are generated by Canadian wastewater treatment facilities annually, which is an increase of 70% from 2009 data (Banga et al. 2009; CCME 2013). Biosolids use as a fertility amendment makes land application seem like an ideal alternative to traditional methods such as incineration and landfilling to manage the large amount of biosolids produced each year (Cooper 2005; Goodman and Goodman 2006). However, land application of biosolids can be hampered by public acceptance or negative perceptions (Goodman and Goodman 2006). For example,

over 1 million tonnes of P fertilizer, as P_2O_5, are applied in Canada (Heffer and Prud'homme 2009), while total P content from biosolids could account for nearly 2% of P fertilizer consumption (Hedley and McLaughlin 2005).

Oversupply of fertilizer P in agricultural soils can cause P accumulation and increase the risk of P leaching and runoff into ground and surface waters (Chambers et al. 2001). Excess P in aquatic ecosystems can lead to conditions that cause the overgrowth of algae and other aquatic plants, which is known as eutrophication (Hutchinson 1969). More efficient P management in agricultural ecosystems requires a better understanding of P forms, transformations, and availability, especially in biosolid-amended soils. Although many researchers have studied the effects of applying biosolids to land (Lu and O'Connor 2001; Maguire et al. 2001; Akhtar et al. 2002), no studies are available that have examined the influence of continuous Alkaline Treated Biosolids (ATB) land application on P dynamics in acidic soils.

The purpose of this study is to examine changes in soil total P and soil available P pools, including Mehlich 3 and water extractable P, as well as plant P uptake, relative to either annual or a single application of an ATB into an acidic agricultural soil in Nova Scotia. This study will examine whether soil P saturation indices, extractable P, and plant uptake P increase as a function of frequency or rate of ATB applications, as well as determining the potential risk of P leaching and runoff from ATB-amended soils.

1.2 Literature review

1.2.1 Biosolids

1.2.1.1 Background information of biosolids

In the United States, the Clean Water Act (CWA) requires municipalities to only discharge treated wastewater (U.S.EPA 1993). The primary purposes of wastewater treatment are to ensure the treated water is safe for reintroduction into water bodies and the solids produced during the treatment process can be recycled or disposed of according to government regulations or guidelines (Chambers et al. 1997). The sewage generated from domestic wastewater contains a broad range of components, including household hazardous waste, pharmaceuticals, pesticides, human excrement, detergents, oil, grease, soaps, shampoos and food stuffs. On-site wastewater treatment such as septic tanks, cesspools and portable toilets are used to treat domestic wastewater at the source while municipal systems often include more levels of treatment, including primary, secondary, and advanced wastewater treatment processes (Figure 1.1). Each level of treatment can greatly improve the quality of both liquid and solid products (U.S.EPA 1993).

The terms "sludge" or "sewage sludge" are generally used to describe the suspended or dissolved solids from the wastewater treatment process, and are easily confused with the term "biosolids". The raw sludge requires additional treatment processes to reduce pathogens, heavy metals, and other contaminants for beneficial use and this treated material is called a "biosolids" (Nova Scotia Department of Environment 2010). Biosolids can be defined as "the nutrient-rich organic materials resulting from the treatment of sewage sludge. When treated and processed, sewage sludge becomes biosolids that can be safely recycled and applied as fertilizer to sustainably improve and maintain productive

soils and stimulate plant growth" (U.S.EPA. 2013). Over 2.5 million wet tonnes of biosolids and sludge are produced by Canadian wastewater treatment facilities every year (CCME 2013). In 2006, approximately 79.3% of wastewaters generated municipally in Canada received secondary or higher treatment before discharging into water bodies (Statistics Canada 2010). Biosolids consist of organic compounds, a variety of micro- and macronutrients, microorganisms, heavy metals and micropollutants (Singh and Agrawal 2008). Based on the nutrients and the organic constituents, biosolids can be used as a soil fertility amendment (Elliott and O'Connor 2007). They can be used in agricultural production systems, home gardens, silviculture, greenways, recreational areas and reclamation of deteriorated sites.

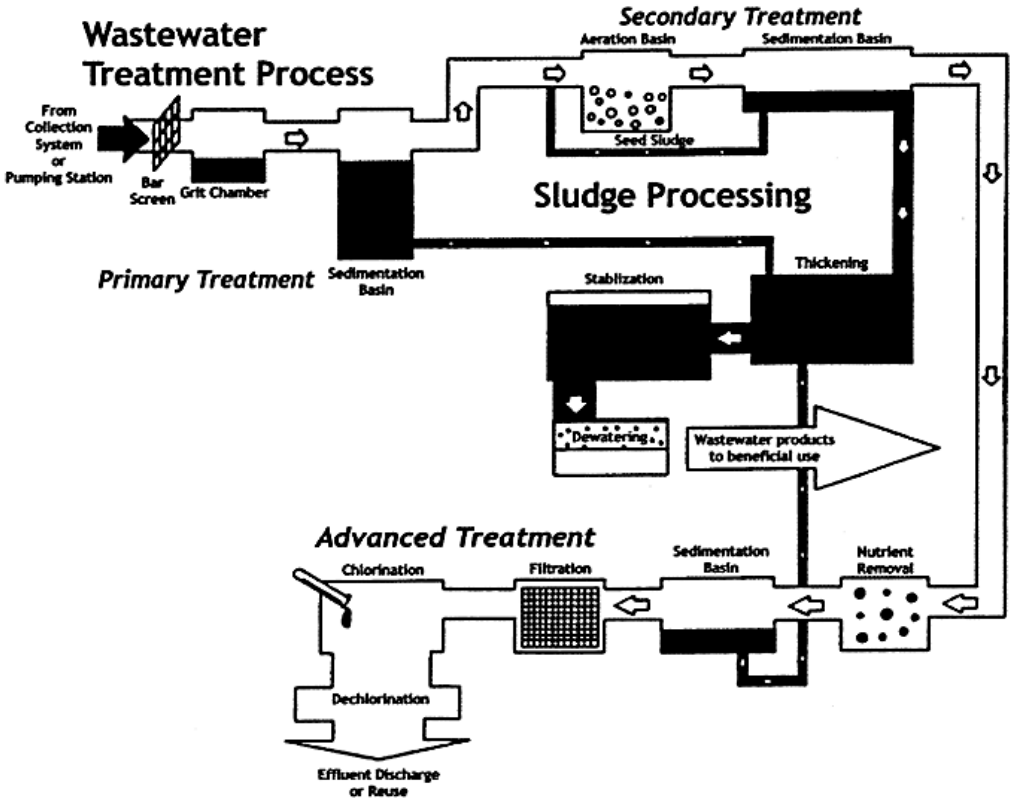

Figure 1.1 Schematic diagram of wastewater treatment facility (Haering et al. 2006)**.**

There are several common approaches to use or dispose of biosolids, including incineration, landfilling, surface disposal, disposal on dedicated sites, and land application to agricultural or non-agricultural lands. Among all the methods mentioned above, approximately 66% of biosolids were applied to agricultural lands to enhance the growth of agricultural crops in the U.S, and approximately the same proportion in Canada (U.S.EPA 2000b). Different forms of biosolids need to be applied through different methods. Liquid forms of biosolid, containing only 3 to 6% total solids and a high percentage of water (94 to 97%), are injected into the soil or applied on the soil surface. Dewatered biosolids can reduce the total volume of biosolids generated, saving on the transportation costs, as well as making handling easier. Dewatered biosolids are typically applied to the soil surface and then incorporated into the soil through disking or plowing (U.S.EPA 2000a).

In the U.S., land application is regulated by the 40 *Code of Federal Regulations* (CFR) Part 503 to ensure the safe use or disposal of the biosolids (U.S.EPA 1994). Unlike the U.S.A., some biosolid regulations or guidelines have been developed at the provincial and territorial level in Canada (CWWA 2012). However, the Canadian Council of Ministers of the Environment (CCME) has national guidelines that many provinces have begun to incorporate into their regulatory frameworks. The guidelines for land application of biosolids in Nova Scotia, which regulate all biosolids for land application in the province, require that they must be treated by one of the approved stabilization methods (Nova Scotia Department of Environment 2010). These methods include composting, alkaline stabilization, aerobic and anaerobic digestion, heat drying and treatment, and pasteurization (Nova Scotia Department of Environment 2010). In Nova Scotia, biosolids

have been divided into two categories, Class A or Class B, based on their heavy metal concentrations and pathogen content to regulate land application requirements. Class A biosolids are treated and stabilized products which meet a higher regulatory standard for heavy metals, pathogens, and priority pollutants. Compared with Class A biosolids, Class B biosolids contain higher levels of metals, pathogens and contaminants (Pepper et al. 2008). Land application of Class B biosolids requires an approval in conformity to section 23 of the Activities Designation Regulations whereas Class A biosolids are allowed unrestricted use as a soil amendment (Nova Scotia Department of Environment 2010).

1.2.1.2 Benefits and risks of biosolids land application

Land application of biosolids has many well documented potential benefits, such as enhancement in soil fertility, soil physical conditions, soil bulk density, porosity, cation exchange capacity, water retention capacity, N and P availability, soil microbial activity and biomass, as well as soil hydraulic conductivity (Akhtar et al. 2002; Singh and Agrawal 2008; Esteller et al. 2009;).

However, biosolids application is still a contentious public issue due to several potential risks, such as toxic metals, organic contaminants, and offensive odors (Krach et al. 2008; Clarke and Smith 2011; LongHua et al. 2012). Although biosolids may be a potential carrier of pathogens, land application of biosolids has been reported to be a low risk activity impacting human health (Zerzghi et al. 2010; Yen-Chih Chen et al. 2011). Beyond all these concerns, nutrient content is regarded as the biggest challenge for sustainable land application of biosolids, especially biosolid P which may limit the long-term ability to continuously apply biosolids (Stehouwer et al. 2000). Eutrophication is a

process of nutrient enrichment that can cause several negative impacts on all types of water bodies such as decreased aesthetic appeal, reduced biodiversity, and threaten human and animal health (Hutchinson 1969).

1.2.1.3 Alkaline stabilization of biosolids

Biosolids need to be stabilized through one of the approved methods before land application in Nova Scotia (Nova Scotia Department of Environment 2010). Alkaline stabilization is one approved method for biosolids treatment, combining high pH (>12) and temperature (between 52 and 62°C), and drying through mixing the sewage sludge and an alkaline admixture (AA) to kill pathogens. A number of industrial by-products have been applied alone or in combination with the biosolids in the treatment process as alkaline reagents, including limestone, cement kiln dust (CKD), lime kiln dust (LKD), lime (CaO), carbide lime, hydrated lime, fly ash, wood ash, and other coal burning ashes (Logan and Harrison 1995; U.S.EPA 2000b). The selection of the alkaline material is commonly based on its local availability and cost. Alkaline stabilization can be an economical wastewater stabilization method with the availability of low cost AA. Generally, alkaline stabilization biosolids meets the minimum requirements to be classified as Class A biosolids. When the pH is maintained at 12 or over for more than 72 hours and temperature is kept at 52°C for at least 12 hours during that time, pathogen kill is considered complete. The material is kept at a pH of 12 for 72 hours and air dried to reach 50% solids (U.S.EPA 2000b).

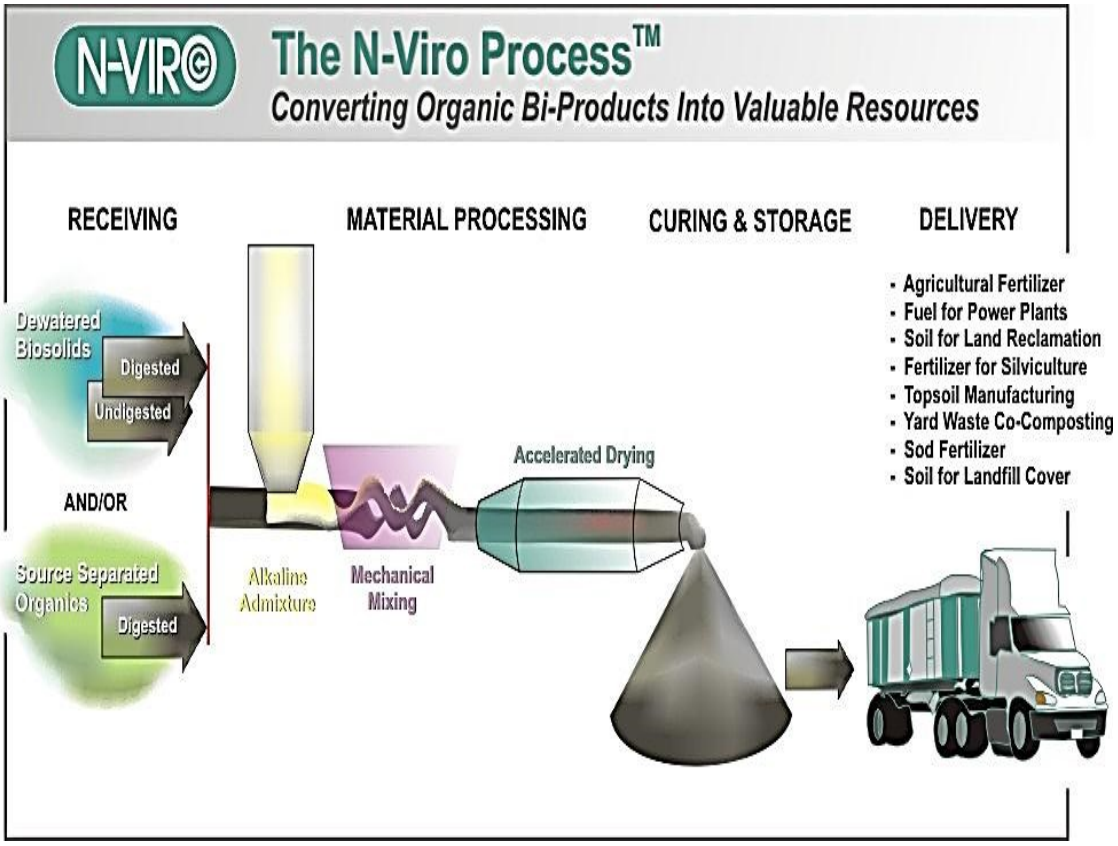

Figure 1.2 Schematic diagram of N-Viro process (N-Viro System Canada LP 2009)**.**

One of the alkaline stabilization technologies is the patented N-Viro process, also known as Advanced Alkaline Stabilization with Subsequent Accelerated Drying (AASSAD) (Hsiau and Lo 1997; N-Viro System Canada LP 2009). This process involves the mixture of dewatered sewage sludge with one or a combination of AA, followed by accelerated drying (Figure 1.2) (Logan and Harrison 1995). In this process, partially dewatered biosolids are mixed with 30% to 40% of AA on a wet basis of biosolids to raise the temperature and pH for pathogen reduction. The mixture is then dried to a total solids content between 60 and 65% to further reduce pathogens. After the drying stage, the mixture is cured for 12 hours in a heat-pulse cell where the temperature is raised to between

52 and 62°C and pH is kept above 12 to stabilize the product and to kill pathogens during storage (Logan and Harrison 1995); N-Viro System Canada LP 2009). The N-Viro product can be used as a lime substitute and fertilizer source based on its physical and chemical characteristics (Logan and Harrison 1995).

1.2.1.4 Phosphorous forms in soil and biosolids

Phosphorus is found in both inorganic and organic P forms. Total P content in the soil can vary from 50 to 1500 mg kg^{-1}, with 50 to 70% of the total P contained in the inorganic forms (Pierzynski et al. 1994). The forms of inorganic P include orthophosphate, pyrophosphate and polyphosphate, while organic P is divided into orthophosphate monoesters, orthophosphate diesters, and phosphonates (Condron et al. 2005). In the soil, the forms of orthophosphate are sensitive to changes in pH. When the pH is below 7.2 but above 2, orthophosphate is found as $H_2PO_4^-$, while HPO_4^{2-} is present when pH is higher than 7.2 but below 12 (Havlin 2005). Both forms of orthophosphate are readily available P forms for plant uptake (Pierzynski et al. 1994).

Phosphorus content in biosolids typically ranges between 2 and 4% on a dry weight basis (Havlin 2005), with 70 to 90% of the total P contained in inorganic forms (Akhtar et al. 2002). Iron phosphates (Fe-P), aluminum phosphates (Al-P) and calcium phosphates (Ca-P) are the dominant forms of inorganic P (Frossard et al. 1996) and most of the Al- and Fe- bounded P are in amorphous forms (Maguire et al. 2001). Water-soluble orthophosphate ranges from 1 to 10 mg L^{-1} in anaerobically digested biosolids (Sommers and Sutton 1980). Most organic P compounds in biosolids are inositol phosphates, diesters of P and phospholipid P (Hedley and McLaughlin 2005). Shober et al. (2006) used X-ray

absorption near-edge structure (XANES) spectroscopy to show that hydroxylapatite, phosphate sorbed to Al hydroxides and ferrihydrite, and phytic acid are the predominant forms of P in lime-treated biosolids. Ajiboye et al. (2007) found through a sequential fractionation method that most of the P in anaerobically digested biosolids are in recalcitrant forms. Differing sources and treatment processes for sewage sludge results in high variability in both P concentrations and forms (Smith et al. 2006) ranging in concentration from <0.1% to over 14% on a dry weight basis (Hedley and McLaughlin 2005).

1.2.2 Phosphorus availability in biosolid-amended soils

Phosphorus is one of the most important macronutrients in crop production (Khare et al. 2004). The term "P availability" is used in agriculture to describe the total available P pool that the plant can directly access and use (Pierzynski et al. 2005). Phosphorus concentrations in plants range from 0.1 to 0.5% (Khare et al. 2004; Havlin 2005) while the total P in surface soil ranges from 0.005 to 0.15%, with approximately 80 to 90% of soil P in non-labile forms, 10 to 20% are in labile P forms, and solution P accounts for only <1% (Smith 1996; Havlin 2005). The availability of P in surface soils can be relatively low, with less than 1% of total P being readily available in some soils (Smith 1996; Havlin 2005).

A large proportion of P in biosolids is in an inorganic form (Akhtar et al. 2002) and the availability of inorganic P in biosolid-amended soils can be affected by climate, vegetation, soil microbial community and the characteristics of both the native and amended P source (Smith et al. 2006). Akhtar et al. (2002) showed that time, soil temperature and moisture influence microorganism activity and P diffusion in soil, so that

soil P cycling and availability can be controlled. Soil characteristics such as pH, clay content, type of clay, Al- and Fe-oxides and hydroxides, and redox potential are other important factors to regulate the availability of P (Khare et al. 2004). Sludge treatment process can also significantly reduce the available forms of P in the sludge (Maguire et al. 2001). Water-soluble orthophosphate can be found at low concentrations in anaerobically digested biosolids (Sommers and Sutton 1980). Various wastewater treatment processes can significantly increase the quantity of Al, Fe and Ca in the biosolids (Hedley and McLaughlin 2005). Calcium-treated biosolids usually have higher P availability than Fe- or Al- treated materials (Hedley and McLaughlin 2005). The application of ATB into acidic soils can result in the transformation from Ca-P to more strongly bonded Al-P and Fe-P forms (Ajiboye et al. 2007) and reduce the availability of P.

Available forms of P in the soil have been traditionally measured through a wide range of chemical extraction methods (Ziadi et al. 2001). However, these soil tests have some limitations in predicting the crop P requirement for optimal yield in the field. Several studies successfully used the electro-ultrafiltration (EUF) technique as an alternative to traditional soil test methods to evaluate short-term and long-term P availability of European and Asian soils (Simard and Sen Tran 1993). The limitation of applying EUF extraction as a soil-testing procedure is the time required for analysis. Some researchers have attempted to use anion exchange membranes (AEMs) to extract the plant available P in the soil (Hernández-Moreno and Negrín 1998; Nuernberg et al. 1998; Cooperband et al. 1999). Montgomery et al. (2005) found predicted P availability determined by in situ AEM method and the P uptake by ryegrass was well correlated in biosolid-amended agricultural soils.

In Nova Scotia, the Mehlich 3 (M3) extraction is the standard soil test P (STP) method applied (Kinley 2005). The Ca-P, as well as Al- and Fe-P can be extracted using the M3 extractant through the function of acetic acid and fluoride compounds (Beegle 2005; Hedley and McLaughlin 2005; Ziadi and Tran 2008). Re-adsorption of M3P back into the soil is a common problem of M3 extractant which can be reduced by the action of fluoride to form a strong complex with Al (Beegle 2005). The correlation relationships between M3 extractant and other test methods have been reported in many studies (Alva 1993; Wang et al. 2004). Phosphorus extracted by M3 has been found to be significantly linearly correlated with Mehlich 2, Olsen, 0.01 M $CaCl_2$, water, Bray 1 and Bray 2 (Lucero et al. 1998; Zbíral and Němec 2002; Wang et al. 2004; Bond et al. 2006). Ziadi et al. (2001) found a close correlation between M3P and EUF, as well as AEMs desorbed P. The significant relationship between plant P uptake, as well as yield in extensive range of soil and M3P were reported by several studies (Piha 1993; Mallarino 2003). Maguire et al. (2001) concluded that the availability of P in biosolids-amended soils can be well correlated by M3P and the molar ratio of P to (Al+Fe) by oxalate extraction.

The extractant solution from the M3 method is traditionally measured through the molybdate-blue colorimetric method that was developed by Murphy and Riley (1962). This method measures the orthophosphate form that reacts with molybdate to turn into a blue color. More recently, many soil testing laboratories have begun to use inductively coupled plasma optical emission spectroscopy (ICP-OES) due to the increasing application of soil test methods, to measure multiple nutrients and increase the efficiency of laboratory analysis (Beegle 2005). Many studies have reported a significant positive relationship between ICP and colorimetric methods, although P concentrations measured by ICP are

usually higher than colorimetric methods (Pittman et al. 2005; Sikora et al. 2005). Ziadi et al. (2009) compared the colorimetric and ICP methods using M3 extracted P from different sites with variety soil properties in eastern Canada and found that ICP P measures were 36% greater than the colorimetric method.

1.2.3 Phosphorus leaching and runoff in biosolid-amended soils

Generally, P concentration in biosolids is approximately half of N and plants require only 10 to 25% as much P as N for growth (Smith 1996). Since biosolids applications are generally based on crop N requirements, P amounts added to soil may be in excess of crop needs (Esteller et al. 2009). Although excessive P is not harmful to the plants, it can be a threat to the quality of surface and ground water systems (Khare et al. 2004; Esteller et al. 2009). Aquatic plant growth will be accelerated when the total P in the streams is over 0.1 mg L^{-1} (EPA standard), which may result in eutrophication (Kinley et al. 2007). Transport of readily available P from Fe- and Al-treated biosolid are reduced due to the low concentrations of extractable P, which make these biosolids have lower leaching potential than the same amount of fertilizer P (Maguire et al. 2001; Elliott et al. 2002; Hedley and McLaughlin 2005). Leaching of P from biosolids treated soils can be determined by several factors, which include soil P sorption capacity, soil hydraulic conductivity, physical forms of biosolids, and soil macropore quantities and continuity (Hedley and McLaughlin 2005).

In some areas of the U.S., site assessment tools have been developed based on the rank of soil vulnerability to P loss in order to generate remedial strategies but biosolids management practices have not been dealt with systematically (Elliott and O'Connor 2007). Although a great deal of soil information can be collected from P sorption isotherms to

estimate P sorption capacity, it is too time consuming and costly for frequent soil testing (Sharpley et al. 2008). Many studies have reported using water extractable phosphorus (WEP) as a fast and simple method to determine P losses from soil amended with both animal manures and biosolids (Brandt et al. 2004; Kleinman et al. 2007). Elliott et al. (2002) suggested using the P saturation index (PSI=P/(Al+Fe)) based on oxalate extraction as the indicator of P leaching risk from biosolid treated soil. Kleinman and Sharpley (2002) showed that soil P sorption saturation (PSI=M3[P/(Al+Fe)]), which is a widely used indicator for soil P runoff and leachate, can be estimated effectively in most soil testing laboratories through M3 extraction in both acidic and alkaline soils. Sims et al. (2002) evaluated 465 soils from the Mid-Atlantic U.S. by using the soil P sorption saturation index and established 5 categories. They suggested that PSI values between 0.06 and 0.11 are the optimum nutrient condition for crop growth with no nutrient additions required and with the least environmental concerns. Soil with PSI values higher than 0.11 were defined as "above optimum", which means soil P would not limit plant growth and no additional P would be recommended. Soil in this category was considered as a significant environmental concern. The other 3 categories included: "below optimum" (PSI < 0.06), "environmental" (PSI > 0.15 to possibly 0.2), and "natural resource conservation" (PSI > 0.2).

1.3 Objective and Hypothesis

The overall objectives of this research project are to:

1) Examine plant P uptake, plant available P, and soil total P as a function of rate and frequency of application of an Alkaline Treated Biosolids (ATB) amended to an agricultural soil over four years.
2) Explore the potential P accumulation in an Alkaline Treated Biosolids (ATB) amended agricultural soil in relation to the rate and frequency of application over four years.

The hypothesis of this study is that long term continuous application of Alkaline Treated Biosolids (ATB) will lead to P accumulation in the soil and increase the available forms of soil P. Soil P saturation index is also hypothesized to increase from long-term continuous Alkaline Treated Biosolids (ATB) applications, which increases the potential for P leaching and runoff in amended soils. Annual Alkaline Treated Biosolids (ATB) will result in higher crop P uptake than single biosolids application.

2 Effect of repeated or single Alkaline Treated Biosolids application on soil P, Al, Fe, pH and P saturation index

2.1 Introduction

Phosphorus (P) is one of the most important but limiting nutrient for plant growth and development. In order to provide sufficient food to feed the fast growing world population, phosphate rock is used extensively to secure the food production (Sattari et al. 2012). Approximately 14.9 million tonnes phosphate rock is used annually for the production of P fertilizer (Cordell et al. 2009). However, reserves of phosphate rock are not infinite resources. Projection of current phosphate rock supply predict peak demand by 2033 and dwindling supplies curbing production thereafter (Cordell et al. 2009). We are approaching a threshold where world agricultural requirements are begin to outpace the available supplies of P which highlights the importance of considering possible alternatives for using P fertilizer produced by phosphate rock.

One of the alternatives is the land application of biosolids, which are the by-product of municipal wastewater treatment that are high in N and P (Warman and Termeer 2005; Cordell et al. 2009). Several research studies report crop yield increases with land application of biosolids (Tiffany et al. 2000; Cooper 2005). However, oversupply of P can easily happen by applying biosolids based on crop N requirements, which results in either potential P loss or excess accumulation in the soils (Shober and Sims 2003). More efficient P management requires a better understanding of the soil P change in biosolids-amended soils.

This chapter discusses the effect of ATB application under two management practices (Annual or single application) on plant available soil P, total soil P and soil pH. Other soil elements such as Al and Fe and their effects on plant available soil P, including

the potential risk of leaching and runoff based on the PSI, are discussed in this chapter as well.

The hypotheses in this chapter include: 1) Annual ATB application will result in P build up in the soil increasing the M3P and WEP levels; 2) Soil pH will increase after annual ATB application in all rates; 3) Both soil extractable Al and Fe level will decrease with the annual application ATB, which will reduce P adsorption; 4) Annual ATB application will increase the risk of leaching and runoff in the amended soil when compared to single ATB application treatments.

2.2 Materials and Methods

2.2.1 Site description and treatments

The study was conducted using soils from Alkaline Treated Biosolids (ATB) amended plots at the Bio-Environmental Engineering Center (BEEC) located in Bible Hill, Nova Scotia (45°23'18" N, 63°14"08" W). This site is a sandy loam soil with a 1% slope, cropped to soybeans in 2008 prior to ATB applications and under continuous corn production after ATB applications in Autumn 2008/Spring 2009 through to 2012.

The recommended rate based on plant nitrogen (N) requirements used for growing corn with ATB was 14 Mg ha^{-1} which was provided by an agronomist working with the biosolids generator, N-Viro Systems Canada LP in Halifax, N.S. Lime was based on the liming recommendation from the Nova Scotia Department of Agriculture Soil Testing Laboratory using a 2007 soil test to bring the soil to a pH of 6.5. The recommended liming rate was equivalent to 2 to 3 Mg ha^{-1} to achieve the target pH. Half rates of ATB and lime were applied to all plots in 2008 fall and 2009 spring. The ATB and lime were applied to

some treatment plots on an annual basis each spring from 2010 to 2012. The ATB chemical properties are shown in Table 2.1 and the detailed field plot application schedule for Alkaline Treated Biosolids is shown in Table 2.2. Inorganic fertilizer was applied annually to all plots, either pre-plant and/or as a side-dress, from 2008 to 2012 based on a soil test to ensure plant responses and germination during the early stages of growth. The K fertilizer regime, using a spring soil test, was for optimal corn yield based on the provincial soil laboratory recommendations. The inorganic nitrogen fertilizer applications were not applied at the provincially recommended levels for optimal corn yield but only as a starter with the seed at rate of 50.4, 25.0, 22.2, and 46.1 kg ha^{-1} in 2009, 2010, 2011, and 2012, respectively. In 2009, a single application of fertilizer P at the rate of and 16.8 kg ha^{-1} was applied to the soil based on the spring soil test.

Table 2.1 Properties of the Alkaline Treated Biosolids (ATB) (Dry basis) applied to the field plots at the Bio-Environmental Engineering center.

Year	2009	2010	2011	2012
Dry Matter (%)	55.0	71.5	69.5	55.9
Nitrogen (%)	1.0	0.8	1.1	1.0
Calcium (%)	23.5	22.0	17.9	21.7
Potassium (%)	0.4	2.1	1.5	1.4
K_2O (%)	0.5	2.6	1.8	1.7
Phosphorus (%)	0.8	0.5	0.7	0.6
P_2O_5 (%)	1.8	1.2	1.6	1.4
Magnesium (%)	0.4	0.5	0.3	0.3
Sodium (%)	0.0	0.2	0.1	0.1
Boron (ppm)	18.9	23.0	19.0	25.8
Copper (ppm)	108.7	85.1	102.2	87.3
Iron (ppm)	5751.1	5647.0	5756.3	6296.7
Manganese (ppm)	408.3	374.9	227.7	207.9
Zinc (ppm)	258.5	179.1	192.2	178.0

Table 2.2 Alkaline Treated Biosolids (ATB) application schedule on field plots at the Bio-Environmental Engineering center.

Date	Activity[1]
Oct 16, 2008	Half rate ATB applied to all plots (i.e. 3.5, 7, 14 and 21 Mg ATB ha^{-1})
May 21, 2009	Half rate ATB applied to all plots (i.e. 3.5, 7, 14 and 21 Mg ATB ha^{-1})
May 13, 2010	Full rate ATB applied to Split #1 (i.e. 7, 14, 21 and 42 Mg ATB ha^{-1})
May 24, 2011	Full rate ATB applied to Split #1 (i.e. 7, 14, 21 and 42 Mg ATB ha^{-1})
May 25, 2012	Full rate ATB applied to Split #1 (i.e. 7, 14, 21 and 42 Mg ATB ha^{-1})

[1] ATB rates are all wet basis.

An 82 m x 60 m field was setup in a split plot design and divided into 48 plots with four blocks to reduce the impact of spatial variability and to isolate a management practice (continuous vs. single ATB applications). The split plot design was used to determine the effect of application rate [a control, a recommended lime (2 to 3 Mg ha^{-1}), 7, 14, 28 and 42 Mg ha^{-1} ATB (wet basis)] and management practice (continuous vs. single ATB applications) on the availability and accumulation of P in the ATB amended soil. The whole plot treatments were two levels of management practices, namely a one-time application in 2008 and 2009 (Management practice #2), or continued applications in 08-09, 09-10, 10-11 and 11-12 (Management practice #1). The sub-plot treatments were the six application rates. The whole plot treatments were randomized within each block, and the sub-plot treatments were randomized within each whole-plot. The experimental units were 12 m x 5 m in size. A guard row measuring 12 m x 2 m was set up between each plot (Figure 2.1).

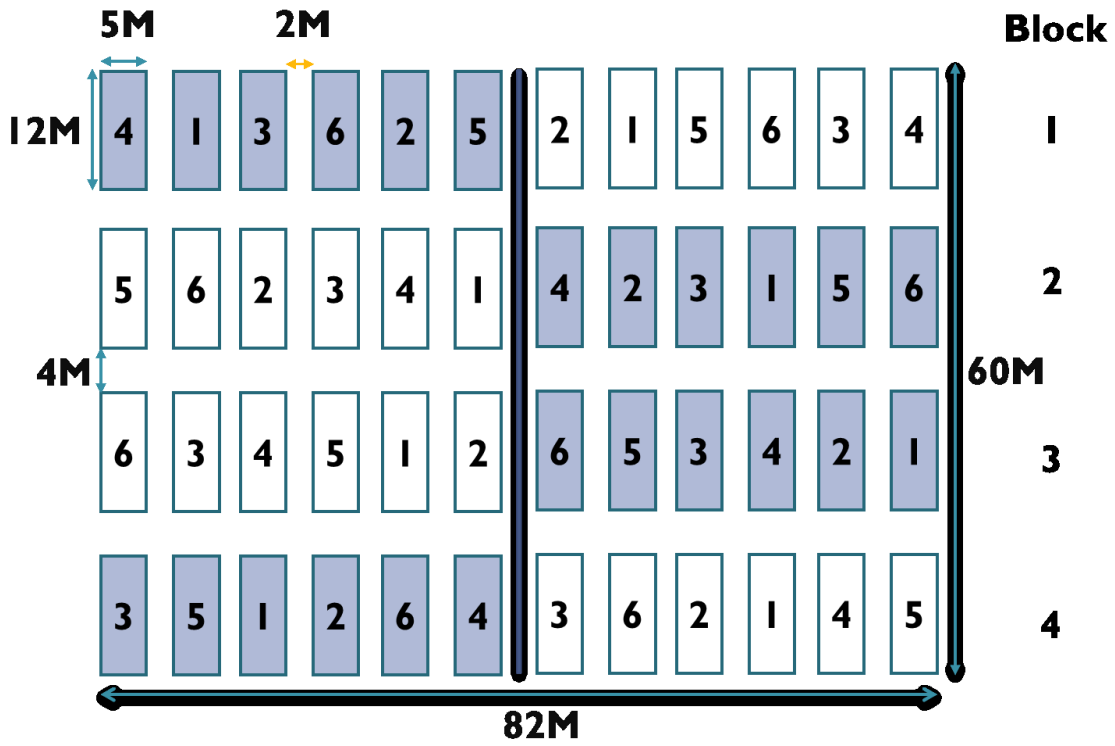

Figure 2.1 Layout of the field experiment design at the Bio-Environmental Engineering center.
[Numbers 1 to 6 refer to the control, recommended lime, 7, 14, 28 and 42 Mg ha^{-1} Alkaline Treated Biosolids (ATB) treatment, respectively; shaded plots represent annual Alkaline Treated Biosolids (ATB) application (Management practice #1), white plots represent single Alkaline Treated Biosolids (ATB) application (Management practice #2)]

2.2.2 Soil sample selection

This study is based on archived soil samples that were collected from the study site between 2008 and 2012. These soil samples were sampled on a monthly basis to a depth of 0 to 30 cm using Oakfield soil probes that were 1.9 cm in diameter and 30 cm in length. Ten soil samples from September 27th and 28th, 2008 were selected as the baseline. Pre-ATB application samples for each year were chosen in order to study the impact of continuous or single application of ATB on P accumulation, availability, and soil P sorption saturation. The sampling date and number of samples for analysis are shown in Table 2.3. The total number of soil samples analyzed in this study was 202 (250 for soil total P test).

Table 2.3 Details of soil sampling dates and number of samples for analysis.

Year	Month	Day	Sampling	Sampling Period	n
2008	9 9	27 28	5	Baseline	10
2009	5 5	7 12	1 (First)	Pre-application	48
2010	4	22	1 (First)	Pre-application	48
2011	5	2	1 (First)	Pre-application	48
2012			1 (First)	Pre-application	48
2013			1 (First)	Pre-application	48

2.2.3 Soil sampling

Original soil samples were based on a composite of six cores per plot from a 0 to 30 cm depth and were collected once each month over the growing season. A fresh subsample was removed for moisture content analysis and the remaining sample was air dried and sieved using a 2 mm stainless steel sieve. The air-dried soil was bagged and stored in a temperature controlled room at 20°C.

2.2.4 Extraction study

Water-extractable phosphorus (WEP) was determined based on the method described by (Sharpley et al. 2008) to determine the readily available forms of P in the ATB-amended soil. Air-dried, sieved (2mm) soil samples (3 g) were mixed in 30 ml of deionized water and shaken at 10 rpm for 1 h. Extracts were then centrifuged at approximately 3000 *g* for 10 min. The solution was filtered through a Whatman No. 42 filter paper. The P concentration in the extract was measured by ICP-OES based on the AOAC official method 985.01 (Horwitz and Latimer 2011). Water-extractable soil P can be calculated from:

Water-extractable soil P (mg P kg $soil^{-1}$)

= [Concentration of P in extract, mg L^{-1}] x [volume of extractant, L/mass of soil, kg] (Sharpley et al. 2008)

A M3 extraction method modified from Sims et al. (2002) was used to extract the M3P, Al, and Fe for determining the available forms of P, as well as soil P sorption saturation (Sharpley et al. 2008) so the potential P leaching and runoff in amended soils can be predicted. A sample (3 g) of air-dried soil was mixed with 30 ml of M3 solution (0.2 *M* CH_3COOH + 0.25 *M* NH_4NO_3+ 0.015 *M* NH_4F + 0.013 *M* HNO_3+ 0.001*M* EDTA) into a 40 mL centrifuge tube. The tubes were placed in a shaker at 10 rpm for 5 min then filtered through Whatman No. 42 filter paper. All the extracts were analyzed for P, Fe, and Al through ICP-OES (Sharpley et al. 2008). For acid soils (pH < 7.0), PSI can be estimated from:

PSI (mmol kg^{-1}) = $\frac{P_{M3}}{Al_{M3}+Fe_{M3}}$ (Sharpley et al. 2008)

2.2.5 Total P digestion for soil samples

Nitric acid digestion method was used to digest the soil samples to analyze the total P. The detailed digestion method was described in the Appendix A. After the digestion, the digested samples were subsequently analyzed without further dilution by ICP-OES based on the AOAC method 985.01 (Horwitz and Latimer 2011) at the Nova Scotia Department of Agriculture Laboratory Services, Bible Hill, NS.

A dry run using reference soil was conducted to test the accuracy of measuring total P by the nitric acid microwave digestion method in soil samples. The reference soil (TILL-2) was collected and characterized in cooperation with the Mineral Resources Division,

Minerals and Continental Geoscience Branch, Geological Survey of Canada. The reference soil used in this study was collected near Scission's Brook, New Brunswick.

2.2.6 Statistical analysis

Statistical analysis for M3P, M3Fe, M3Al, WEP, soil Total P (TP), soil TP ratio, PSI, and pH measured in 2009 in an agricultural soil cropped to corn, where the previous crop was soybean and no management differences existed, was as a randomized complete block design with eight blocks. The same response variables measured from 2010 to 2012, including 2013 for TP in soil, where the crops were also corn, were analyzed using a repeated measures ANOVA in a split-plot design with four blocks. The two factors of interest, management practices and ATB rates, were considered to be fixed, while block and management practices x block were considered to be random. The variance-covariance matrix of the residuals, selected based on the basis of Akaike's Information Criterion, was variance components (VC).

The model adequacy was tested prior to ANOVA to determine if the assumption of normality was violated, thereby invalidating the F-test. To check whether the assumption of normality was satisfied, the Normal Probability Plot of residuals was plotted with the residuals calculated based on the formula $e_{ij} = y_{ij} - \bar{y}_{i.}$, where y_{ij} is the observation j in treatment i and $\bar{y}_{i.}$ is the corresponding treatment average. If the p-value was greater than 0.1, the normality assumption was considered satisfied. If the assumption of normality was violated, a power transformation was conducted on the original data and the residuals were calculated again for normality checking (Montgomery 2009). For unbalanced designs (the number of observations taken within each treatment is different in a single-factor

experiments), the consequence of constant variance violation is more serious than in the balanced designs. The constant variance was checked through plotting the Scatterplot of Residuals vs Fits. If the residuals showed an impression of a horizontal band, the assumption of constant variance was considered to be met. If this assumption was violated, a power transformation was conducted on the original data and the residuals were calculated again for testing constant variance (Montgomery 2009). In this study, the square root transformation and square transformation were conducted on the soil total P (Soil TP) and water extractable P (WEP) data, respectively.

If the data met the requirement of model adequacy test, a repeated measures analysis of variance (ANOVA) was completed for each response variable using the Mixed Procedure of SAS v.9.3. If significance was found on the effects (p<0.05), a further multiple means comparison was completed by comparing the Least Square Means of corresponding treatment combinations. The letter groupings were generated using a 5% level of significance for both main and two-factor interaction effects. Linear correlation/regression analyses were performed to find out the relationship among available soil P, soil total P, pH, M3Al and M3Fe, PSI and cumulative ATB applied over four years.

2.3 Results

2.3.1 Effect of Alkaline Treated Biosolids under both management practices on soil test P and total P

2.3.1.1 ANOVA result for soil test P

Table 2.4 ANOVA *p*-values for the main and interaction effects of year, management practice and Alkaline Treated Biosolid (ATB) rates on Mehlich 3 P (M3P) (kg P ha^{-1}), Water Extractable P (WEP) (kg P ha^{-1}), Soil Total P (TP) (kg P ha^{-1}) and Soil Total P (TP) ratio from 2010 to 2012 (2013 for Soil TP and soil TP ratio).

	M3P	WEP	Soil TP	Soil TP ratio
Year	0.314	**<.0001**	**<.0001**	0.675
Management practices	0.184	0.017	0.488	0.923
Year x Management practices	0.510	0.141	0.342	**<.0001**
ATB rates	<.0001	0.005	**0.001**	0.001
Year x ATB rates	0.966	0.886	1.000	0.987
Management practices x ATB rates	**0.035**	**0.000**	0.165	**0.012**
Year x Management practice x ATB rates	0.432	0.789	0.791	0.651

*Significant effects that needed multiple means comparison are shown in bold.

The ANOVA analysis (Table 2.4) shows that no 3-way interaction effects were detected for each of the response variable, but M3P, WEP and soil TP ratio measured from 2010 to 2012 (2013 for soil TP ratio) had highly significant interaction effects of management practices x ATB rates. Soil TP was had significant main effects of ATB rates and year. The WEP fraction in soil also had a significant main effect of year. The soil TP ratio also displayed a significant interaction effect of year x management practices. The comparison of treatment means through the Least Square Means approach was conducted for each significant response.

2.3.1.2 Mehlich 3 extractable P (M3P)

No treatment effect was detected in 2009 for M3P, which resulted in the average of 366.1±66.2 kg P ha^{-1} (n=48). The M3P concentrations measured under the annual 28 and 42 Mg ha^{-1} ATB treatments from 2010 to 2012, were significantly higher than the rest of the annual ATB treatments, or control and lime treatments (Table 2.5). The M3P measured under both 7 and 14 Mg ha^{-1} ATB were not significantly different from control. Lime was not statistically different from the control and 7 Mg ha^{-1} ATB. For single ATB treatments over the corresponding period, no significant differences were detected among lime, 14, 28, and 42 Mg ha^{-1} ATB (Table 2.5). Both lime and 7 Mg ha^{-1} ATB were not statistically different from the control. When comparing across the management practices, the M3P concentrations measured after applying 28 and 42 Mg ha^{-1} ATB annually were not statistically different from the single 14, 28 and 42 Mg ha^{-1} ATB applications. Annual applications of 14 Mg ha^{-1} ATB resulted in lower M3P concentrations than annual high rates of ATB application but were not higher than control and the entire single ATB treatment concentrations. The M3P concentrations measured under 7 Mg ha^{-1} ATB, under different management practices, were not statistically different from each other or from the control but were lower than the concentrations of both annual and single high rates of ATB application rates. For the lime treatments, M3P concentrations measured under single application were higher than annual application but the concentrations of M3P from both lime treatments were not different from control (Table 2.5). A significant positive linear relationship was observed (R^2=0.637, p<0.05) between M3P and P added from ATB under annual application over 2010 to 2012, with an increase of 0.23 kg M3P ha^{-1} with each kg addition of ATB (Figure 2.2).

Table 2.5 Effect of Management practice x Alkaline Treated Biosolids (ATB) rates interactions on Mehlich 3 extractable P (M3P) (kg P ha^{-1}) from 2010 to 2012.

Management practices	ATB rates (Mg ha^{-1}) [1]	M3P (kg P ha^{-1})
Annual	0	358.8±52.1 CD
	7	321.8±62.7 D
	14	396.2±61.1 BC
	28	460.8±60.6 A
	42	439.9±51.6 AB
	LIME	310.1±125.9 D
Single	0	347.0±35.9 CD
	7	354.9±44.2 CD
	14	416.5±94.0 AB
	28	424.4±78.3 AB
	42	424.3±81.9 AB
	LIME	385.7±59.2 BC

*Values are presented as means±SD (n=12). Means followed by the same letter are not significantly different. The letter grouping is based on LSMeans (p<0.05).
[1] ATB rates are all wet basis.

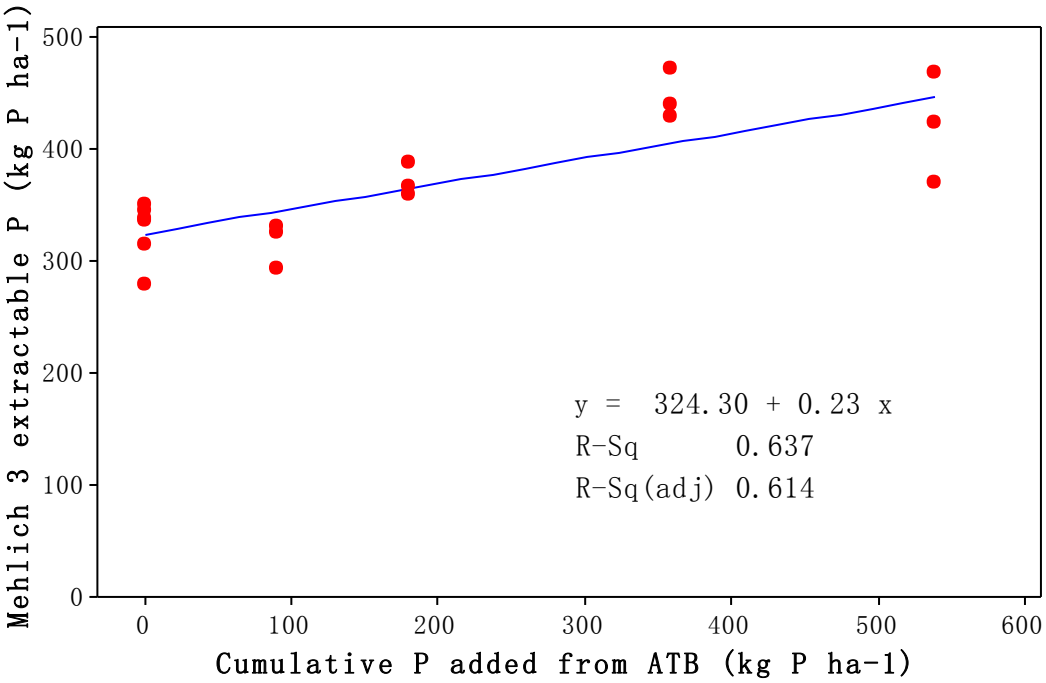

Figure 2.2 The relationship between Mehlich 3 extractable P (M3P) (kg P ha^{-1}) and cumulative P added from annual Alkaline Treated Biosolids (kg P ha^{-1}) application from 2010 to 2012.

2.3.1.3 Water extractable P (WEP)

In contrast with M3P, the WEP concentrations measured under all ATB rates and management practices were within a narrow range from 10.8±0.6 to 14.2±0.6 kg P ha^{-1}. The WEP concentrations measured among 14, 28 and 42 Mg ha^{-1} ATB within the annual ATB application were not significantly different but were higher than the control and most single application treatments (Table 2.6). No statistical differences in WEP were detected among lime, 7 and 42 Mg ha^{-1} ATB within the annual ATB application. Under single ATB treatments, only the 7 Mg ha^{-1} ATB had significantly higher WEP concentrations. Across the management practices, annual ATB application resulted in higher WEP than single application within most of the ATB rates except control and 7 Mg ha^{-1} ATB (Table 2.6). No treatment effect was detected in 2009 for WEP, which resulted in the average of 13.3±0.3 kg P ha^{-1} (n=48).

Table 2.6 Effect of Management practice x Alkaline Treated Biosolids (ATB) rates interactions on water extractable P (WEP) (kg P ha^{-1}) from 2010 to 2012.

Management practices	ATB rates (Mg ha^{-1})[1]	WEP (kg P ha^{-1})
Annual	0	12.0±0.6 CD
	7	12.6±0.6 BC
	14	14.2±0.6 A
	28	13.9±0.7 A
	42	13.4±0.5 AB
	LIME	12.7±0.6 BC
Single	0	11.7±0.5 CD
	7	12.7±0.4 BC
	14	11.7±0.5 CD
	28	11.9±0.5 CD
	42	10.8±0.6 D
	LIME	11.4±0.5 D

*Values are presented as means±SD (n=12). Different letters indicate the significant differences based on LSMeans (p<0.05).
[1] ATB rates are all wet basis.

The WEP measured across management practices and ATB rates in 2010 and 2012 was not different from each other but higher than 2011 (Figure 2.3). Moreover, no significant relationships were detected between M3P and WEP, across both management practices, during 2010 to 2012 (Figure 2.4).

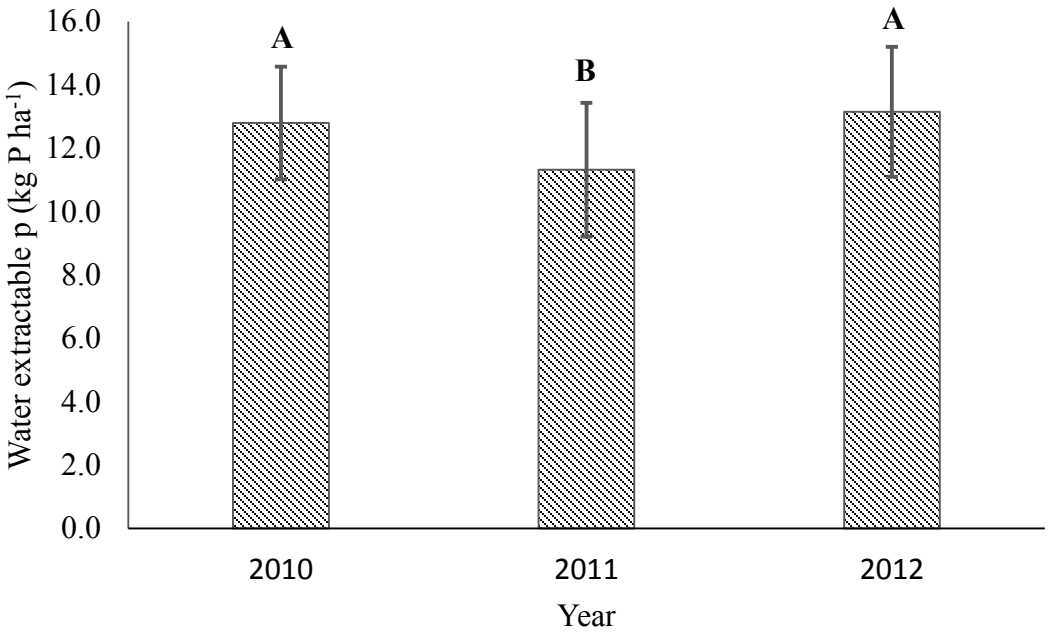

Figure 2.3 Main effect of year on Water extractable P (WEP) (kg P ha^{-1}) measured over both management practices and all Alkaline Treated Biosolids (ATB) rates in 2010, 2011, and 2012.

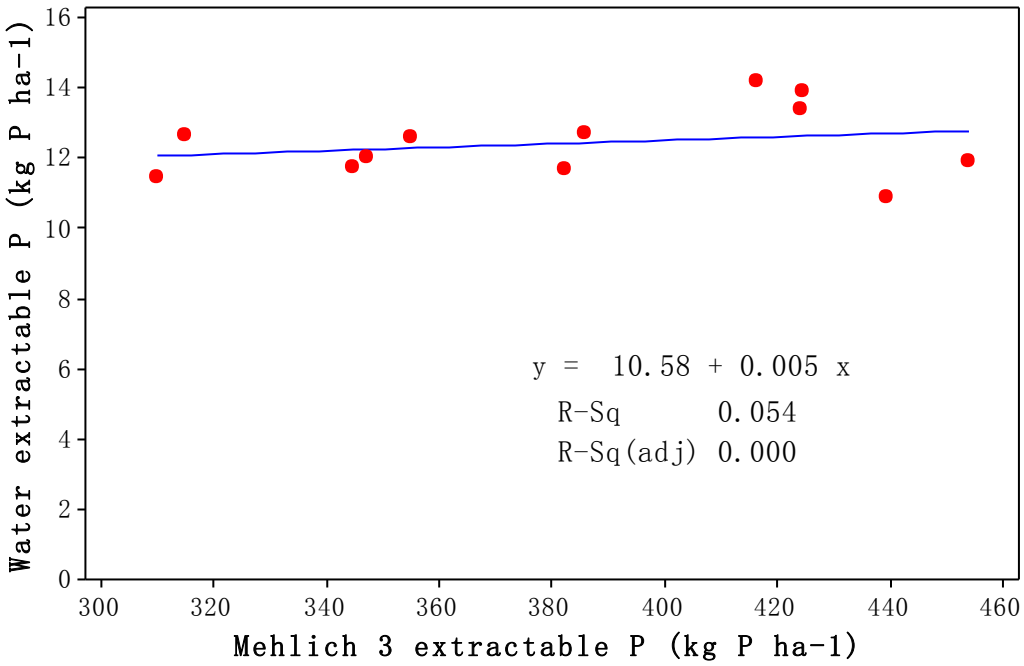

Figure 2.4 The relationship between Mehlich 3 extractable P (M3P) (kg P ha^{-1}) and water extractable P (WEP) (kg P ha^{-1}) under both management practices from 2010 to 2012.

2.3.1.4 Total P (TP) in soil

Although no management practices and ATB rate interaction effects were detected from 2010 to 2013, a significant effect on soil TP of increasing ATB rates was observed (Table 2.7). Soil TP measured across management practices at the 14, 28 and 42 Mg ha^{-1} ATB rates showed no differences from each other but were significant higher than the control and 7 Mg ha^{-1} ATB. Lime treatment was not statistically different compared to ATB rates on soil TP (Table 2.7).

Table 2.7 Effect of Alkaline Treated Biosolids (ATB) application rates over both management practices on Soil Total P (TP) (kg P ha^{-1}) from 2010 to 2013.

ATB rates (Mg ha^{-1})[1]	Soil TP (kg P ha^{-1})
0	2425.5±66.9 B
7	2415.2±72.7 B
14	2654.8±94.9 A
28	2667.7±83.6 A
42	2693.6±96.9 A
LIME	2553.8±108.5 AB

*Values are presented as means±SD (n=32). Means followed by the same letter are not significantly different. The letter grouping is based on LSMeans (p<0.05).
[1] ATB rates are all wet basis.

A significant effect of year was also observed over the study period for soil TP measured in both management practices and for ATB rates (Figure 2.5). In 2012, significantly higher soil TP concentrations were measured followed by 2011 and 2013.

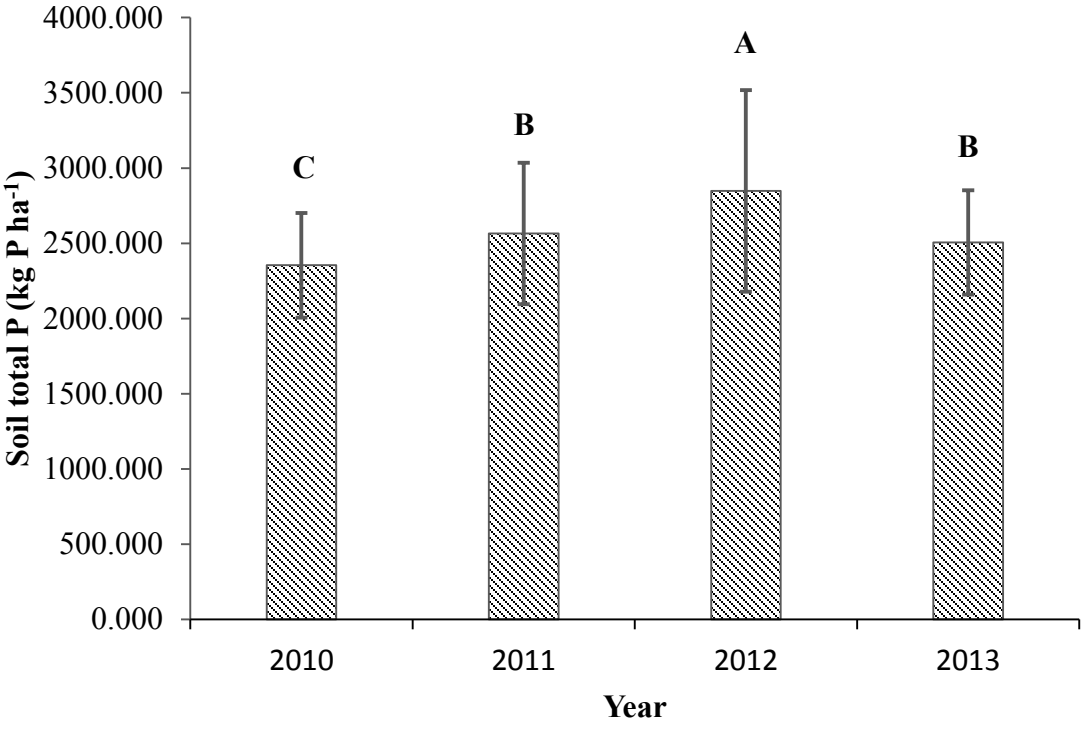

Figure 2.5 Main effect of year for Soil Total P (TP) (kg P ha^{-1}) measured over both management practices and all Alkaline Treated Biosolids (ATB) rates in 2010, 2011, 2012 and 2013.

To examine changes in soil TP over time, soil TP was expressed as a ratio of the ATB treatment over the control for the corresponding year. The interaction effect of year x management practices on the soil TP ratio from 2010 to 2013 is shown in Table 2.8. No remarkable differences were detected among years on soil TP ratio under either annual or single ATB application except 2013. Management practice effects were not observed for the soil TP ratio measured in 2010, 2011 and 2012 but in 2013 annual ATB applications resulted in a greater soil TP ratio (Table 2.8).

Table 2.8 Effect of Year x Management practice interactions on soil Total P (TP) ratio from 2010 to 2013.

Management practices	Year	Soil TP ratio[1]
Annual	2010	1.01±0.13 BC
	2011	1.04±0.16 BC
	2012	1.07±0.13 ABC
	2013	1.16±0.16 A
Single	2010	1.11±0.18 AB
	2011	1.09±0.14 AB
	2012	1.11±0.21 AB
	2013	0.99±0.07 C

*Values are presented as means±SD (n=20). Different letters indicate the significant differences based on LSMeans (p<0.05).
[1] Soil TP ratio = Soil TP ATB treatment/soil TP unamended control.

The management practice x ATB rate interaction for soil TP ratio resulted in no significant differences between 14, 28 and 42 Mg ha^{-1} ATB in either management practice (Table 2.9). Under the annual ATB applications, these same rates were greater than the 7 Mg ha^{-1} ATB and the lime. In contrast, under the single ATB application the three highest rates were not significantly different than the lime. When comparing the same ATB rates over the two management practices no significant differences were observed for the soil TP ratio. A significant positive linear relationship was detected between M3P and soil TP (R^2=0.831, p<0.05) with a slope of 0.324 (Figure 2.6).

Table 2.9 Effect of Management practices x Alkaline Treated Biosolids (ATB) rates interactions on Soil Total P (TP) ratio from 2010 to 2013.

Management practices	ATB rates (Mg ha^{-1})[1]	Soil TP ratio[2]
Annual	7	0.98±0.10 CD
	14	1.08±0.11 AB
	28	1.17±0.12 A
	42	1.13±0.21 AB
	LIME	0.98±0.11 CD
Single	7	1.01±0.08 BD
	14	1.11±0.20 AC
	28	1.05±0.16 ABC
	42	1.11±0.17 AC
	LIME	1.09±0.17 ABC

*Values are presented as means±SD (n=16). Different letters indicate the significant differences based on LSMeans (p<0.05).

[1] ATB rates are all wet basis.

[2] Soil TP ratio = Soil TP ATB treatment/soil TP unamended control.

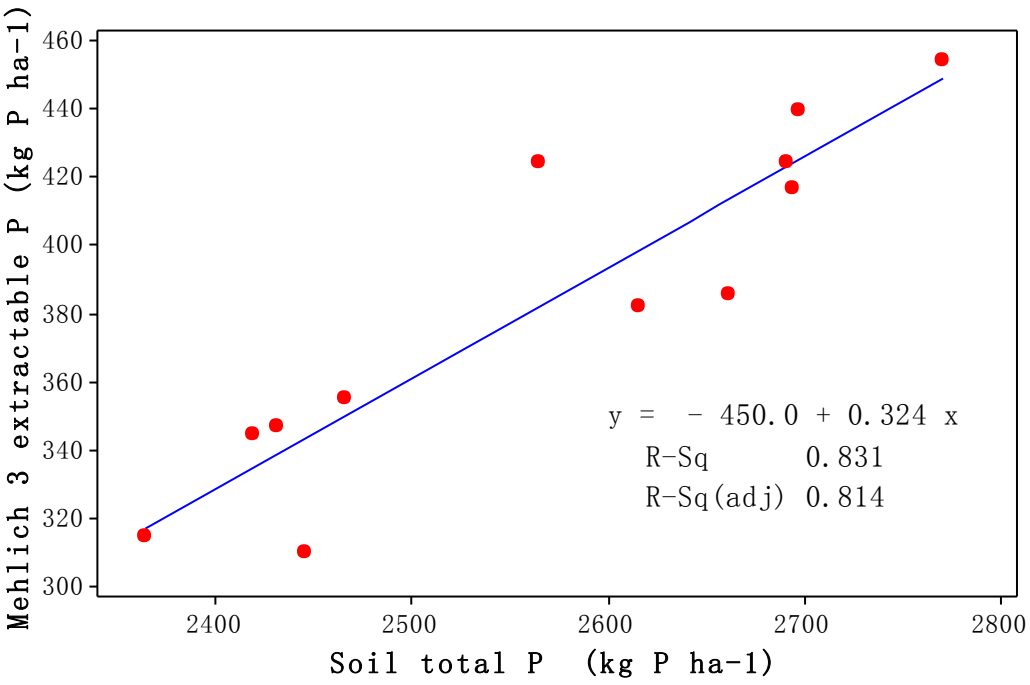

Figure 2.6 Relationship between Mehlich 3 extractable P (M3P) (kg P ha^{-1}) and soil total P (soil TP) (kg P ha^{-1}) under both management practices from 2010 to 2012.

2.3.2 Effect of Alkaline Treated Biosolids under both management practices on soil pH, Al, Fe and P saturation index

2.3.2.1 ANOVA result for soil pH, Al, Fe and P saturation index

The ANOVA analyses indicates that the ATB rates had a significant effect on all the responses measured in 2009, except M3Fe (Table 2.10). The ANOVA analyses (Table 2.11) showed that soil pH and PSI measured from 2010 to 2012 were highly affected by the three way interaction of year x management practice x ATB rates. The M3Fe and M3Al were affected by the two-way interaction of management practices x ATB rates, as well as the main effect of year. Comparison of the treatment means for the response variables are described in the following sections.

Table 2.10 ANOVA *p*-values for the Alkaline Treated Biosolids (ATB) rates effects on soil pH, Mehlich 3 Fe (M3Fe) (kg Fe ha^{-1}), Mehlich 3 Al (M3Al) (kg Al ha^{-1}) and P saturation index (PSI) in 2009.

	pH	M3Fe	M3Al	PSI
ATB rates	**<.0001**	0.6694	**0.0244**	**0.0423**

*Significant effects that need multiple means comparison are shown in bold.

Table 2.11 ANOVA *p*-values for the main and interaction effects of Management practice and Alkaline Treated Biosolids (ATB) rates on soil pH, Mehlich 3 Fe (M3Fe) (kg Fe ha^{-1}), Mehlich 3 Al (M3Al) (kg Al ha^{-1}), P saturation index (PSI) from 2010 to 2012.

	pH	M3Fe	M3Al	PSI
Year	<.0001	**0.003**	**<.0001**	0.303
Management practices	0.036	0.948	0.490	0.296
Year x Management practices	<.0001	0.220	0.246	0.190
ATB rates	<.0001	<.0001	<.0001	<.0001
Year x ATB rates	0.011	0.973	0.949	0.938
Management practices x ATB rates	<.0001	**0.002**	**0.000**	0.006
Year x Management practice x ATB rates	**0.001**	0.975	0.171	**0.033**

*Significant effects that needed multiple means comparison are shown in bold.

2.3.2.2 Soil pH

Soils with greater ATB application rates had higher soil pH when compared to those at the low ATB rates and lime in 2009 (Table 2.12). The pH was highly affected by the three way interaction of year x management practices x ATB rates based on the data shows in Table 2.13. For comparing the soil pH measured under each of the ATB rate across year, all the annual ATB application rates, except control showed a trend of increasing over three years. This trend was not detected under the single ATB application (Table 2.13). For the soil pH measured within each year, higher ATB rates, under either annual or single ATB applications in 2010, 2011, and 2012, resulted in increased soil pH. In 2010, applying each ATB rate annually resulted in no difference on soil pH than one-time corresponding ATB rate of application. However, annual ATB application in 2011 and 2012 resulted in higher soil pH than corresponding single application ATB rates (Table 2.13). A significant positive linear relationship was observed between the amount of ATB applied and soil pH under annual ATB treatment (R^2=0.085, $p<0.05$) with a slope of 0.012 (Figure 2.7).

Table 2.12 Effect of different Alkaline Treated Biosolids (ATB) application rates on soil pH in 2009.

ATB rates (Mg ha^{-1})[1]	pH
0	5.34±0.06 C
7	5.43±0.05 BC
14	5.54±0.09 B
28	5.80±0.09 A
42	5.75±0.09 A
LIME	5.34±0.09 C

*Values are presented as means±SD (n=8). Means followed by the same letter are not significantly different ($p<0.05$). The letter grouping is based on LSMeans.
[1] ATB rates are all wet basis.

Table 2.13 Effect of Year x Management practice x Alkaline Treated Biosolids (ATB) rates interactions on soil pH from 2010 to 2012.

Management practices	ATB rates (Mg ha^{-1})[1]	Year		
		2010	2011	2012
Annual	0	A 5.3±0.1 gh	A 5.3±0.3 ef	A 5.2±0.2 g
	7	B 5.8±0.1 cde	B 5.9±0.1 c	A 6.1±0.1 cd
	14	C 5.8±0.3 cd	B 6.1±0.1 b	A 6.5±0.2 b
	28	B 6.4±0.1 ab	B 6.4±0.2 a	A 6.8±0.1 a
	42	C 6.3±0.1 ab	B 6.6±0.1 a	A 6.8±0.1 a
	LIME	B 5.5±0.3 fg	AB 5.7±0.2 cd	A 5.6±0.2 e
Single	0	A 5.2±0.2 h	A 5.2±0.4 f	A 5.4±0.3 fg
	7	A 5.6±0.2 def	A 5.6±0.3 d	A 5.5±0.2 ef
	14	A 5.9±0.3 c	A 5.7±0.3 cd	A 6.0±0.1 d
	28	A 6.2±0.2 b	A 6.2±0.2 b	A 6.3±0.3 bc
	42	A 6.5±0.3 a	B 6.1±0.3 b	AB 6.2±0.1 bc
	LIME	A 5.6±0.3 ef	A 5.7±0.4 cd	A 5.6±0.3 e

*Values are presented as means±SD (n=4). Means followed by the same letter within each ATB rate over years (upper case) or within each year over ATB rates and management practice (lower case) are not significantly different (p<0.05). The letter grouping is based on LSMeans.

[1] ATB rates are all wet basis.

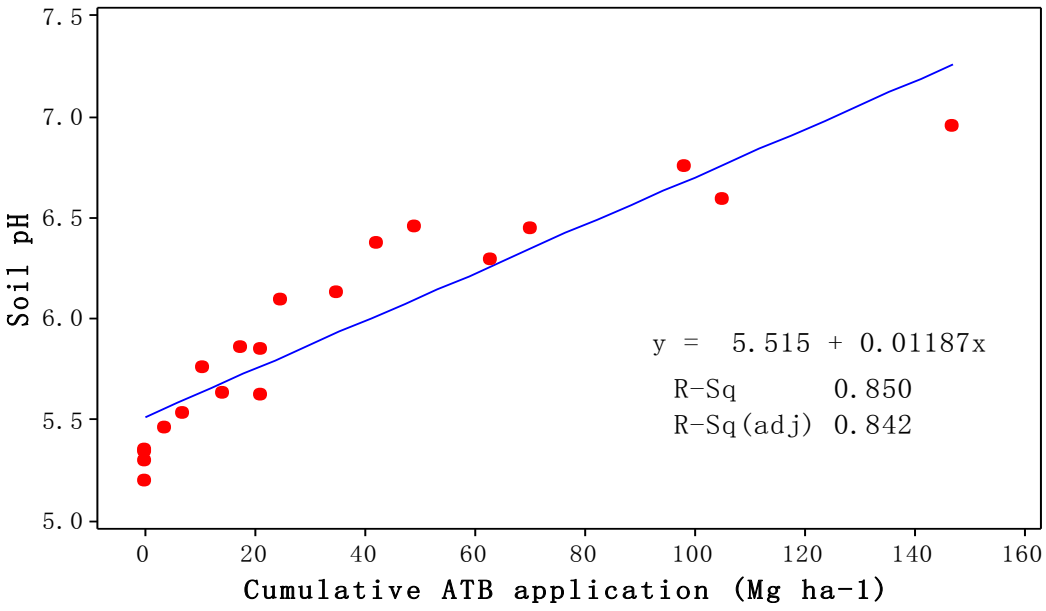

Figure 2.7 The effect of annual Alkaline Treated Biosolids (ATB) application (2009 to 2012) on soil pH.

2.3.2.3 Mehlich 3 Extractable Al and Fe (M3Al and M3Fe)

The ATB rates affected M3Al concentrations but not M3Fe concentrations in 2009 (Table 2.14). Control plots resulted in significantly higher M3Al than most of the ATB application plots ($p < 0.05$) but were not statistically different from lime and 14 Mg ha^{-1} ATB. No significant differences were detected among ATB rates on M3Al. The M3Al concentration measured under lime was not statistically different from any ATB rates except 28 Mg ha^{-1} ATB (Table 2.14).

High rates of ATB application resulted in significantly lower M3Fe concentrations than lime and low rates of ATB application treatments ($p < 0.05$) under annual ATB application (Table 2.15). For the single ATB application, no statistical differences were detected within ATB rates. Overall, the M3Fe concentrations measured under single ATB application were not different from annual application treatments within each treatment. The statistical analysis for M3Al showed a similar trend as M3Fe (Table 2.15). Overall, higher ATB application rates resulted in lower M3Fe and M3Al concentrations.

Table 2.14 Effect of Alkaline Treated Biosolids (ATB) application rates on Mehlich 3 Fe (M3Fe) (kg Fe ha^{-1}) and Mehlich 3 Al (M3Al) (kg Al ha^{-1}) in 2009.

ATB rates (Mg ha^{-1})[1]	M3Fe (kg Fe ha^{-1})	M3Al (kg Al ha^{-1})
0	1426.1±99.8 A	7148.3±2091 A
7	1473.2±100.0 A	6836.9±210.8 BC
14	1502.9±129.5 A	6936.6±252.9 ABC
28	1431.1±114.6 A	6734.2±214.0 C
42	1373.3±113.8 A	6873.1±241.1 BC
LIME	1529.1±102.1 A	7040.8±206.5 AB

*Values are presented as means±SD (n=8). Means followed by the same letter in a column are not significantly different (p<0.05). The letter grouping is based on LSMeans.

[1] ATB rates are all wet basis.

Table 2.15 Effect of Management practice x Alkaline Treated Biosolids (ATB) rates interactions on Mehlich 3 Fe (M3Fe) (kg Fe ha^{-1}) and Mehlich 3 Al (M3Al) (kg Al ha^{-1}) from 2010 to 2012.

Management practices	ATB rates (Mg ha^{-1})[1]	M3Fe (kg Fe ha^{-1})	M3Al (kg Al ha^{-1})
Annual	0	1537.2±56.3 ABCD	6735.3±95.3 ABC
	7	1363.8±44.1 EFG	6621.0±100.1 ABC
	14	1401.0±58.2 ABCDE	6392.9±99.0 DF
	28	1257.2±71.1 FH	6178.3±117.5 E
	42	1095.1±72.7 GI	6186.6±141.2 E
	LIME	1444.1±55.4 ABCDE	6775.8±91.1 ABC
Single	0	1274.0±101.9 CDHI	6961.5±130.9 A
	7	1414.0±78.0 ABEFG	6660.5±96.1 BD
	14	1388.9±115.4 ABCEFG	6643.1±138.6 BCD
	28	1341.9±95.9 BCDGH	6556.9±121.6 BCDE
	42	1236.2±98.9 DHI	6462.4±149.8 CEF
	LIME	1514.9±53.3 AEF	6577.7±98.6 BCDE

*Values are presented as means±SD (n=12). Means followed by the same letter in a column (over both management practices) are not significantly different (p<0.05). The letter grouping is based on LSMeans.

[1] ATB rates are all wet basis.

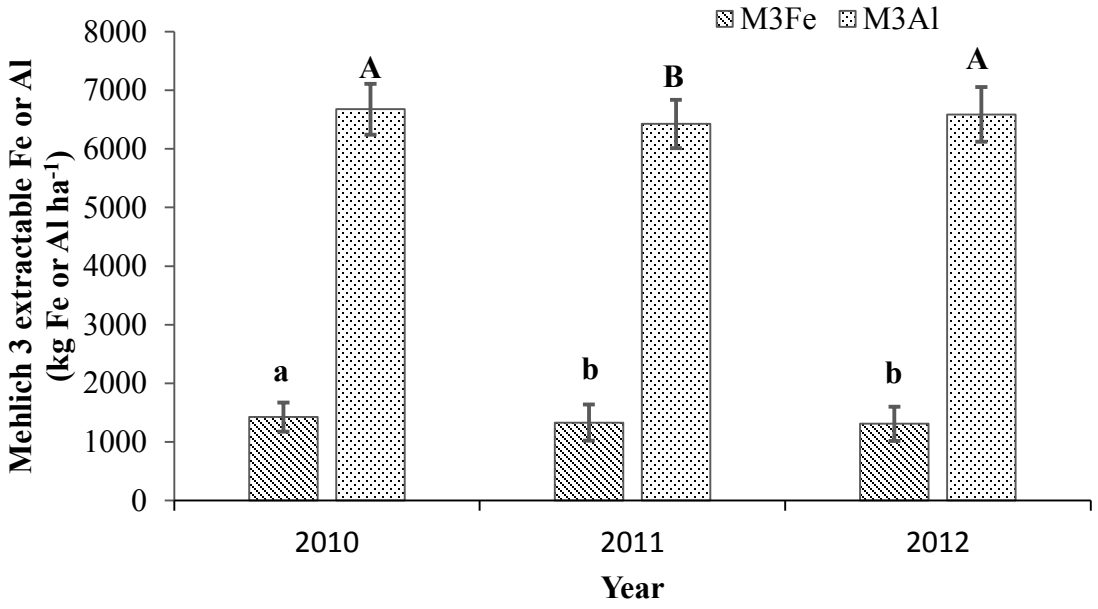

Figure 2.8 Main effect of year on Mehlich 3 extractable Fe and Al (M3Fe or M3Al) (kg Fe or Al ha^{-1}) measured over both management practices and Alkaline Treated Biosolids (ATB) rates in 2010, 2011 and 2012.

The M3Fe showed a weak but significant negative linear relationship with increasing soil pH from 2010 to 2012 under both management practices (R^2=0.471, $p<0.05$) (Figure 2.9). The M3Al had a similar linear relationship with pH as M3Fe, but with a higher coefficient of determination (R^2=0.842, $p<0.05$) (Figure 2.10). The M3Al had a significant negative linear relationship with M3P as well, even though the R^2 was lower (R^2=0.568, $p<0.05$) (Figure 2.11). However, no relationship was detected between M3Fe and M3P.

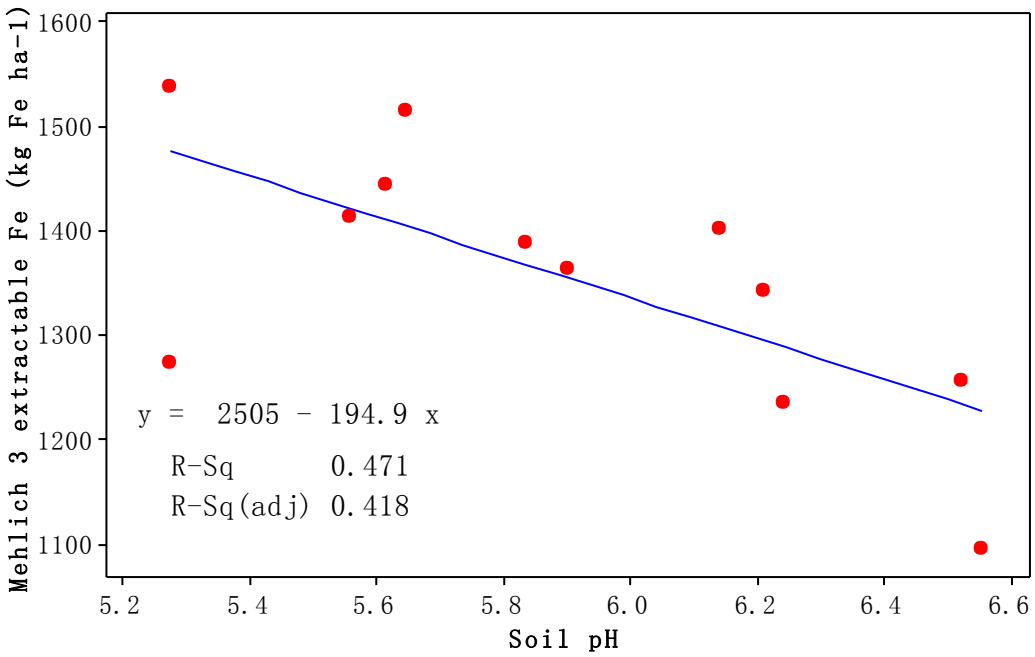

Figure 2.9 The relationship between Mehlich 3 Fe (M3Fe) (kg Fe ha^{-1}) and soil pH under both management practices from 2010 to 2012.

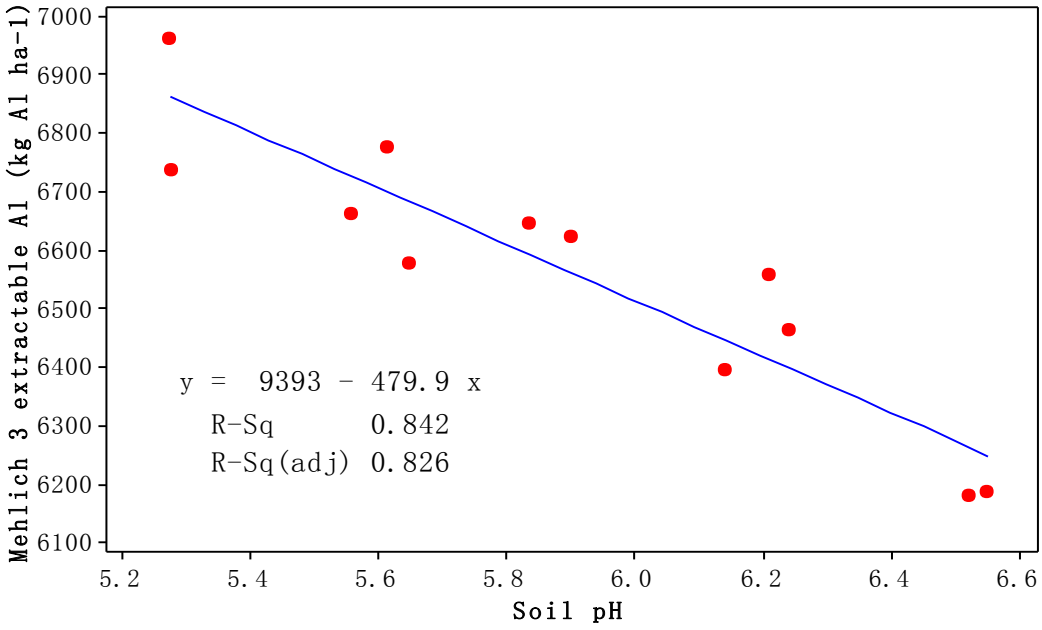

Figure 2.10 The relationship between Mehlich 3 Al (M3Al) (kg Al ha^{-1}) and soil pH under both management practices from 2010 to 2012.

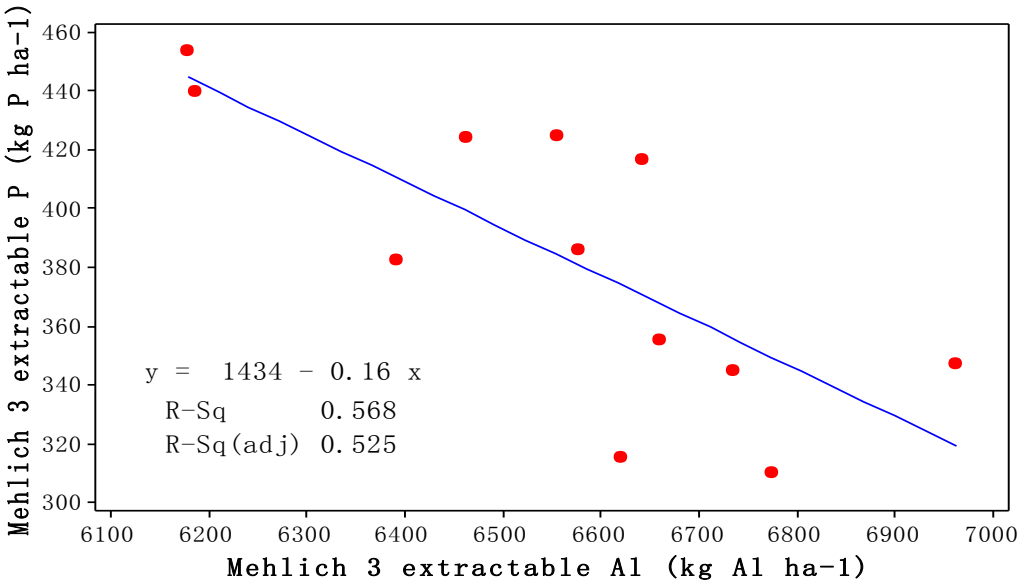

Figure 2.11 The relationship between Mehlich 3 extractable Al (M3Al) (kg Al ha^{-1}) and Mehlich 3 extractable P (M3P) (kg P ha^{-1}) under both management practices from 2010 to 2012.

2.3.2.4 P saturation index (PSI)

The PSI was significantly higher for the 28 Mg ha^{-1} ATB rate than other ATB rates, except in the 14 Mg ha^{-1} ATB, in 2009 (Table 2.16). The PSI was highly affected by the three-way interaction of year x management practices x ATB rates (Table 2.17). The only significant difference in PSI within each ATB rate over the three years was observed in the 42 Mg ha^{-1} ATB treatment. Under annual applications at this rate PSI increased over time, while under the single ATB application the PSI decreased. No other ATB rate over year interaction was observed over both management practices. Within each year, increasing ATB rates under annual applications resulted in higher soil PSI values. While a similar increase in PSI occurred in 2010 under the single application when greater quantities of ATB were applied, this effect was not observed in either 2011 or 2012. No significant relationship was detected between WEP and PSI in this study, but significant relationship was detected between PSI and cumulative P added from ATB (R^2=0.72, P<0.05) (Figure 2.12).

Table 2.16 Effect of different Alkaline Treated Biosolids (ATB) application rates on P saturation index (PSI) in 2009.

ATB rates (Mg ha^{-1})[1]	PSI
0	0.044±0.003 B
7	0.042±0.002 B
14	0.045±0.003 AB
28	0.050±0.004 A
42	0.043±0.003 B
LIME	0.040±0.003 B

*Values are presented as means±SD (n=8). Means followed by the same letter are not significantly different. The letter grouping is based on LSMeans (p<0.05).
[1] ATB rates are all wet basis.

Table 2.17 Effect of Year x Management practice x Alkaline Treated Biosolids (ATB) rates interactions on P saturation index (PSI) from 2010 to 2012.

Management practice	ATB rates (Mg ha^{-1})[1]	Year		
		2010	2011	2012
Annual	0	A 0.041±0.005 c	A 0.043±0.003 def	A 0.041±0.004 cde
	7	A 0.040±0.006 c	A 0.042±0.004 ef	A 0.037±0.004 de
	14	A 0.046±0.006 bc	A 0.052±0.007 abcde	A 0.046±0.005 cd
	28	A 0.061±0.006 a	A 0.059±0.007 ab	A 0.060±0.004 ab
	42	B 0.047±0.003 bc	A 0.060±0.002 a	A 0.067±0.004 a
	LIME	A 0.041±0.008 c	A 0.039±0.006 f	A 0.034±0.010 e
Single	0	A 0.043±0.002 c	A 0.042±0.003 ef	A 0.042±0.003 cde
	7	A 0.044±0.003 bc	A 0.046±0.004 cdef	A 0.042±0.002 cde
	14	A 0.054±0.007 ab	A 0.049±0.006 bcdef	A 0.052±0.005 bc
	28	A 0.054±0.006 ab	A 0.056±0.006 abc	A 0.051±0.006 bc
	42	A 0.063±0.009 a	AB 0.054±0.004 abcd	B 0.050±0.001 c
	LIME	A 0.045±0.005 bc	A 0.054±0.003 abc	A 0.044±0.003 cde

*Values are presented as means±SD (n=4). Means followed by the same letter within each ATB rate over years (upper case) or within each year over ATB rates and management practice (lower case) are not significantly different (p<0.05). The letter grouping is based on LSMeans.

[1] ATB rates are all wet basis.

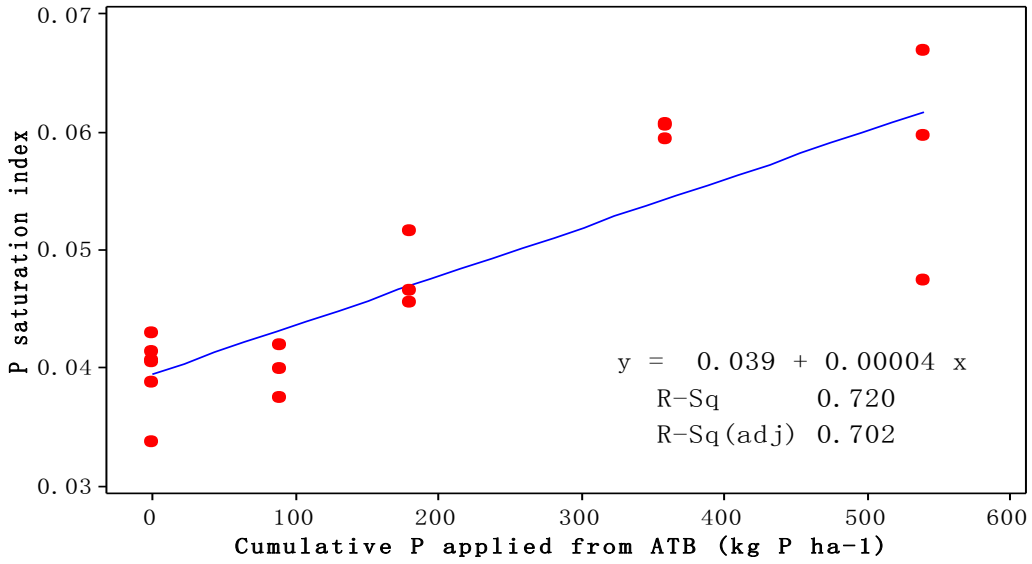

Figure 2.12 The relationship between P saturation index (PSI) and Cumulative P applied from Alkaline Treated Biosolids (ATB) under annual Alkaline Treated Biosolids (ATB) application from 2010 to 2012.

2.4 Discussion

2.4.1 Effect of Alkaline Treated Biosolids under both management practices on soil test P and total P

The M3P concentrations measured in all the control ATB and lime application plots from 2010 to 2012 under both management practices ranged from 310.1±125.9 to 460.8±60.6 kg P ha^{-1}. Based on the Nova Scotia Department of Agriculture soil fertility ratings, these values are considered to be in the Excessive range when measured by ICP (Nova Scotia Department of Agriculture 2011). The high M3P measured throughout the study is a result of high soil P at the site (346.8±21.0 kg P ha^{-1}). Applying 14, 28 and 42 Mg ha^{-1} ATB annually did not increase the M3P significantly across three years in this study relative to the single application of ATB. This result is different from what we expected. For annual applications of 14, 28 and 42 Mg ha^{-1} ATB, a total of 179.7, 359.3 and 539.0 kg P ha^{-1} were added to the soil from 2010 to 2012. Soil storage is one possible avenue for the added P to be located. On the basis of soil TP measured, higher ATB rates resulted in higher soil TP concentrations, however this occurred regardless of management practices. Furthermore, while soil TP also increased over the study period, no differences between management practices were observed. The other possible location for the added P from the annual ATB applications is in the plant biomass and grain or losses through runoff and leaching. The following chapter will examine the crop P uptake dynamics more closely but an increase in uptake was observed under the annual ATB application, in both the biomass and grain, with increasing ATB rates. Variability at the research site resulting differences in topography or drainage may also explain some of the anomalous result observed over the study period. No appreciable changes in M3P were detected for each ATB rates across three years under single application, which is consistent with the result reported by Smith

et al. (2005) who applied inorganic fertilizer and poultry litter into high soil test P (STP) soils and found the change of M3P over 16 weeks incubation study was not significant. This result may be due to the high variability in the data which masked any changes that may have resulted from the added P from annual ATB applications. Significant ATB rate effects on M3P under annual applications were evident at the high ATB rates in both management practices although no effect of year was observed (Table 2.5). These results indicate that the hypotheses should be rejected and suggest that there may be little difference in how management practice affects plant available P in soils receiving ATB that are already naturally high in either soil TP or M3P. Based on the M3P data measured in this study, it is possible that the M3P extraction method is not a suitable measure of P availability in high STP soils receiving certain organic amendments.

The slope of 0.23 based on the significant relationship between M3P and cumulative P added from ATB means that 4.34 kg P ha^{-1} from ATB would be required to increase M3P by 1.0 kg (Figure 2.2). This result is a bit higher than Zhang (2006), who reported that approximately 3.57 kg ha^{-1} was required to increase M3P by 1.0 kg based on a long-term triple super phosphate (0-46-0) application study. However, this value was two times lower than the result reported for biosolids by Schroder et al. (2008). The difference may be due to the different biosolids used in each study or could be due to differences in P saturation in the soils. The biosolids used by Schroder et al. (2008) were neutral with a median pH as 6.5, which is approximately half of the pH for the ATB used in the present study. The application of ATB increased the soil pH, which decreased the exchangeable Al and Fe, while Schroder et al. (2008) found that biosolids had no effect on soil pH and increased the concentration of oxalate Al and Fe that increase the P adsorption in the

amended plots. These results suggest that ATB used in this study may be less effective than inorganic P fertilizer in increasing available soil P but better than biosolids that do not have a liming effect like ATB.

The WEP measured in 2009 under all the ATB rates was 3.4 kg P ha^{-1} higher than the initial soil WEP (9.9±0.5 kg P ha^{-1}). Bond et al. (2006) explored the change of WEP concentration by applying P fertilizer to soils with different initial M3P and found that WEP was elevated significantly regardless of the P application rate. For the average WEP measured across the period 2010 to 2012, 14, 28 and 42 Mg ha^{-1} ATB applied annually increased WEP by 2.5, 2 and 2.6 kg P ha^{-1} higher than corresponding single ATB rates. This result indicates that applying high ATB rates annually can elevate the most labile P fraction more than one-time high ATB rates application, which will potentially increase the risk of losses of P through leaching and possibly runoff. No relationship was detected between M3P and WEP in this study (Figure 2.4). However, many other studies found a strong linear relationship between M3P and WEP (Fuhrman 2005; Bond et al. 2006). This may due to the narrow range of WEP detected in this study across all the ATB rates and management practices resulting from the use of colorimetric rather than ICP.

No significant trend of soil TP increasing was observed in each ATB rates under both management practices across the period 2010 to 2013 was observed, which indicates that P accumulation was not apparent after four years of study. This is surprising because a large amount of P was applied to the annual ATB application plots, particularly at 28 and 42 Mg ha^{-1} ATB. These two ATB rates contributed 420 and 631 kg P ha^{-1} in total from 2009 to 2013. These results suggest that high variability in this site may mask the changes that occurred in this study or added P was not being retained in the soil with high initial

STP. There are some indications that it may have been taken up by the crop plants or possibly lost in runoff. The soil TP showed an overall trend of increasing with more applied P over 2010 to 2013 regardless of the management practices (Table 2.7). Similar trend was also observed under the annual ATB application for the soil TP ratio over 2010 to 2013 (Table 2.8 and 2.9), while the ATB rates effect was not observed on soil TP ratio under the single ATB application. These result suggest the potential soil P accumulation under the high rates of ATB application. Although no P accumulation was observed in the present study, it can be expected that soil TP will accumulate under the annual high rates of ATB application in the longer-term study. The slope of 0.324 based on the significant relationship between M3P and soil TP indicates that the increase of 3.1 kg ha^{-1} soil TP will elevate 1 kg ha^{-1} M3P (Figure 2.6). Soil TP was highly correlated with M3P in this acidic soil receiving alkaline treated biosolids which has potential as a management tool.

2.4.2 Effect of Alkaline Treated Biosolids under both management practices on soil pH, Al, Fe and P saturation index

The slope of 0.012 based on the relationship between the amount of ATB applied and soil pH under annual ATB treatment means each Mg ha^{-1} ATB application would be required to increase soil pH by 0.012 unit at the 0-30cm depth (Figure 2.7). Similar relationships between soil pH and ATB were reported by Sloan (1995) and Cooper (2005) as well. The soil pH measured under the control plots was 5.28, which was below the critical level (pH 5.5) at which crop growth would be limited due to Al toxicity (Kisinyo 2014). The lime and ATB application under both management practices increased the soil pH level above pH 5.5, which reduced the risk of Al toxicity. In Nova Scotia, the optimum pH level for corn production is 6.5 or over (Nova Scotia Federation of Agriculture 2006). In this study,

the soil pH reached the optimum pH level only with the annual 28 and 42 Mg ha^{-1} ATB treatments (Table 2.13). The soil pH measured in each ATB rate under single ATB application showed the residual effect across three years. Cooper (2005) reported that ATB had the same long-term effect as agricultural lime on soil pH. The results in this study show that ATB application elevated pH regardless of the management practices from 2009 to 2012. Overall, these results suggest that ATB can be used as the substitute for agricultural lime and long-term high rates of ATB application are necessary to elevate acidic soils to the optimum pH level for maximum crop yield.

The results for M3Fe and M3Al showed a trend of decreasing with the applied ATB increase under the annual ATB application over three years and not under the single ATB application. This result indicates that ATB application is the main reason of the M3Fe and M3Al decrease over three year period. Iyamuremye and Dick (1996) also found that increasing rates of manure application reduced the extractable Al and increased the extractable P at the same time. The significant relationship between soil pH and M3Fe as well as M3Al suggest that the change of soil pH due to the application of ATB is the main reason for the decrease of soluble Fe and Al in this study. The relationship between M3P and M3Al indicates that the decrease of soluble Al due to the increase of soil pH can increase the P availability in this soil. The increase of soil pH due to the ATB application reduces the possibility of P sorption with Fe and Al oxides as $AlPO_4$ and $FePO_4$ and increase the solubility of Al-P and Fe-P (Iyamuremye and Dick 1996; Havlin 2005).

The ATB rates effect was observed for each year under the annual ATB application but this effect was not detected under the single ATB application. These results suggest that higher soil available P from annual high rates of ATB application will increase the risk

of P runoff with a longer-term study because the P is more saturated in this soil. The significant relationship observed between PSI and cumulative P added from ATB under annual application with a slope of 0.00004 (Figure 2.12) was similar to the relationship found by Schroder et al. (2008) that oxalate based PSI was highly correlated with P supplied by biosolids (R^2=0.86). The slope of 0.00004 indicate that 250 kg ha^{-1} P from ATB would be required to increase the PSI by 1%. However, the highest PSI in this study was measured under the annual 42 Mg ha^{-1} ATB application in 2012 (PSI=0.067), which was considered as the optimum nutrient condition for crop growth with no nutrient additions required and with the least environmental concerns based on the categories developed by Sims et al. (2002). In this present study, the 42 Mg ha^{-1} ATB contributed only an average of 172.5 kg P ha^{-1} every year which suggests that the PSI in this site will take a long time to reach the level of "above optimum" (PSI_{M3}>0.11), even with the application of this ATB rate annually. The PSI measured under annual low rates and single full rates of ATB application were generally lower than 0.06, which were under the category of "below optimum". It suggests low P related environmental concerns based on the current ATB application strategy in this site.

2.5 Conclusions

This study was conducted in a soil with low pH and excessive P. The high initial STP in the soil resulted in high M3P measured in all the ATB and lime application plots across the four-year study. The M3P concentrations measured under all the ATB rates regardless of management practices were consistent over four years. The M3P results were highly variable in the high STP soils receiving range of ATB applications over several years

suggesting this test for plant available P and PSI may not be suitable. Other factors including time of sampling and losses through leaching or runoff may have also played a significant role in the variability in results measured in this study. In contrast to the results observed for M3P, management practices resulted in higher WEP under the annual high rates ATB application. No relationship was detected between WEP and M3P. The ATB rate effects on soil TP were found under annual application. Although no P accumulation was observed in the present study, it can be expected that soil P will accumulate under the annual high rates of ATB application after a longer-term study.

The soil pH was significantly increased with the annual ATB application with optimum pH level observed under annual 28 and 42 Mg ha^{-1} ATB applications. The ATB rate effects were also detected under the single application. The ATB used in this study can be used as the substitute of agricultural lime and the lime application rate needs to be adjusted if the ATB was applied. The ATB rates had a significant effect on reducing the M3Al and M3Fe level in the soil under both management practices, with lower exchangeable Al and Fe level detected in higher ATB rates in general. The PSI result shows that current ATB application strategy resulted in low P related environmental concerns even with the annual 42 Mg ha^{-1} ATB application but potential risk of P losses is expected with a longer-term study.

3 Annual corn responses to different Alkaline Treated Biosolids application frequency and rates

3.1 Objective and hypotheses

The objective of this study is to determine corn biomass and grain P uptake from either annual or single applications of ATB at six different application rates over four years (2009 to 2012).

The hypotheses of this study include: 1) highest crop P uptake will be detected under annual high rates of ATB application; 2) single high rates of ATB application will result in improved crop P uptake responses due to residual P contributions from the Alkaline Treated Biosolids (ATB) over four years.

3.2 Material and methods

3.2.1 Site description

The detailed site description was given in Chapter 2.

3.2.2 Plant sample selection

Plant samples, which were separated into corn biomass and grain, collected each year from 2009 to 2012 were analyzed for total P in this study. The total number of plant samples for this study was 384.

3.2.3 Sampling plant samples

The biomass sampling was conducted once per season on plants at full maturity. Each plot had six rows of corn at 77 cm spacing between rows. Four meters of corn were randomly taken (1 m from each of four rows in the middle) within each plot (Figure 3.1). The total

weight of the corn plants was determined and then the cobs were removed and weighed separately. Subsamples of the whole plant biomass and the grain were taken for analysis for moisture content and nutrient analysis. The plant samples were dried in an oven at 55 °C for 48 h or until they reached a constant weight and then were ground using a Wiley mill with a 2 mm screen. All the plant samples were further ground with Retsch M200 ball mill to pass through a 0.425 mm screen. The ground samples were stored in the capped vials in a temperature controlled room at 20 °C.

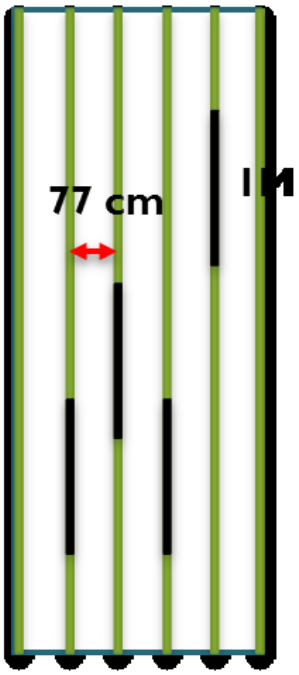

Figure 3.1 Plant sample collection from treatment plots.

3.2.5 Total P digestion for plant samples

A microwave assisted nitric acid digestion method was used to digest the plant samples for total P analysis. Air dried biomass or grain samples, ground to <0.425 mm particle size, were measured out to approximately 0.25 g onto a Whatman #41 70mm Ø ashless filter

paper and the plant sample weights were recorded to 4 decimals places. The details of running the microwave assisted nitric acid digestion and sample analysis were described in Chapter 2.

3.2.6 Climatic conditions

Both the temperature and precipitation in Truro, NS during the growing seasons from 2009 to 2012 are shown in Table 3.1. The mean monthly temperature was warmer than 30 years average most years in most months in this study. The precipitation showed great variability across the four years. Overall, the total precipitation during the growing seasons from 2009 to 2011 increased each year but a decrease was measured in 2012. The 2009, 2010 and 2012 seasons were 18.6%, 10%, and 13.4% drier than the 30 year average, respectively, while the 2011 season was 6.9% wetter than the average.

Table 3.1 Mean monthly temperature (°C) and precipitation (mm) in Truro during the four year of the experiment (Environment Canada 2013)**.**

Year	May	June	July	August	September	Total for period
			Temperature °C			
2009	10.8	*	17.6	19.9	13.7	NA
2010	11.1	15.2	19.8	19.4	16.3	NA
2011	10.5	13.6	18.8	18.5	16.0	NA
2012	11.8	14.6	20.0	20.7	17.0	NA
Average (30 years)	9.8	15.0	18.6	18.4	14.1	NA
			Precipitation (mm)			
2009	88.6	*	71.0	179.6	73.0	412.2
2010	48.0	99.6	125.2	65.3	117.5	455.6
2011	124.2	144.3	94.3	135.6	43.1	541.5
2012	101.8	75.1	58.5	54.1	148.9	438.4
Average (30 years)	109.7	98.3	102.2	92.7	103.6	506.5

*missing data
NA not applicable

3.2.7 Calculation

Details of the calculations of each response are shown below:

Biomass P uptake (kg P ha^{-1}) = Biomass P (mg kg^{-1}) x 1000 (kg Mg^{-1}) x 10^{-6} (kg mg^{-1}) x biomass yield (Mg ha^{-1});

Grain P uptake (kg P ha^{-1}) = Grain P (mg kg^{-1}) x 1000 (kg Mg^{-1}) x 10^{-6} (kg mg^{-1}) x grain yield (Mg ha^{-1});

3.2.8 Statistical analysis

Statistical analysis for corn biomass and grain yield, as well as corn biomass P uptake (BPU) and grain P uptake (GPU) measured in 2009, where the previous crop was soybean and no management differences existed, was as a randomized complete block design with eight blocks. The same response variables measured from 2010 to 2012, where the crop was corn, was analyzed with a repeated measures ANOVA in a split-plot design with four blocks was used. The two factors of interest, management practices and ATB rates, were considered to be fixed, while block and management practices x block were considered to be random. The variance-covariance matrix of the residuals, selected based on the basis of Akaike's Information Criterion, was variance components (VC). Before the ANOVA analysis, model adequacy test was conducted. Details of model adequacy test were described in Chapter 2.

If the data met the requirement of model adequacy test, a repeated measures analysis of variance (ANOVA) was completed for each response variable using the Mixed Procedure of SAS v.9.3. If significance was found on the effects (p<0.05), a further multiple means comparison was completed by comparing the Least Square Means of

corresponding treatment combinations. The letter groupings were generated using a 5% level of significance for both main and two-factor interaction effects. Linear correlation/regression analyses were performed to find out the relationship among crop yield, crop P uptake, and some responses discussed in Chapter 2.

3.3 Results

3.3.1 ANOVA results

The statistical analysis indicates that the ATB rates had significant effect on corn grain yield and GPU in 2009, but not on corn biomass yield and BPU (Table 3.2). The biomass yield, BPU, BPU ratio, GPU and GPU ratio measured from 2010 to 2012 were highly affected by the two-way interaction of management practice x ATB rates (Table 3.2). The BPU ratio and GPU were also affected by two-way interaction of year x management practice. Both corn biomass and BPU were affected by the main effect of year as well. The three-way interaction of year x management practices x ATB rates had the significant effect on the corn grain yield. The results of the mean comparisons for each response are shown in the specific sections below.

Table 3.2 ANOVA *p*-values for the Alkaline Treated Biosolids (ATB) rates effects on corn biomass (Mg DM ha^{-1}), corn grain (Mg DM ha^{-1}), corn biomass P uptake (BPU) (kg P ha^{-1}) and corn grain P uptake (GPU) (kg P ha^{-1}) in 2009.

	Biomass	Grain	BPU	GPU
ATB rates	0.9037	**0.0004**	0.3861	**0.0056**

*Significant effects that need multiple means comparison are shown in bold.

Table 3.3 ANOVA *p*-values for the Alkaline Treated Biosolids (ATB) rates effects on corn biomass (Mg DM ha^{-1}), corn grain (Mg DM ha^{-1}), corn biomass P uptake (BPU) (kg P ha^{-1}), corn biomass P uptake ratio, corn grain P uptake (GPU) (kg P ha^{-1}) and corn grain P uptake ratio from 2010 to 2012.

	Biomass	Grain	BPU	BPU ratio	GPU	GPU ratio
Year	**<.0001**	<.0001	**<.0001**	0.009	<.0001	0.092
Management practices	0.039	0.008	0.077	0.040	0.017	0.031
Year x Management practices	0.392	0.305	0.159	**0.009**	**0.046**	0.110
ATB rates	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Year x ATB rates	0.539	0.962	0.908	0.514	0.827	0.622
Management practices x ATB rates	**<.0001**	<.0001	**0.000**	**0.007**	**<.0001**	**0.000**
Year x Management practice x ATB rates	0.126	**0.041**	0.191	0.414	0.065	0.188

*Significant effects that needed multiple means comparison are shown in bold.

3.3.2 Crop yield

No significant treatment effect was detected for the year of 2009, which resulted in an average of 16.1±0.3 kg biomass ha^{-1} (n=48). The corn biomass yields measured under all the annual ATB rates except control, lime and 7 Mg ha^{-1} ATB were significantly higher than single ATB treatments within each rate of ATB application (Table 3.4). The corn biomass yields measured under 28 and 42 Mg ha^{-1} ATB were not statistically different from each other, but were significantly higher than rest of the ATB rates under both management practices. Annual 14 Mg ha^{-1} ATB treatment applications resulted in corn biomass yields that were not significantly different from yields for the annual and single 7 and single 42 Mg ha^{-1} ATB applications. Applying 7 Mg ha^{-1} ATB annually resulted in no significant difference from the entire single ATB rates except control and 14 Mg ha^{-1} ATB. With regard to the single ATB application only, high ATB rates did not result in significantly higher biomass yield than low ATB rates. Corn biomass yields measured

under single 14 and 28 Mg ha^{-1} ATB were not significantly different from the control. No statistical differences were detected among annual and single lime application as well as the control on corn biomass yield. All the annual ATB treatments resulted in higher corn biomass yields than the control (Table 3.4). The corn biomass yield measured in year 2010 and 2011 were not different from each other but lower than the year 2012 (Figure 3.2).

Table 3.4 Effect of Management practice x Alkaline Treated Biosolids (ATB) application rates interactions on corn biomass yield (Mg DM ha^{-1}) from 2010 to 2012.

Management practices	ATB rates (Mg ha^{-1})[1]	Biomass (Mg DM ha^{-1})
Annual	0	9.2±0.5 E
	7	10.9±0.6 BC
	14	11.6±0.3 B
	28	13.3±0.5 A
	42	13.6± 0.5 A
	LIME	10.1±0.5 CDE
Single	0	9.6±0.5 DE
	7	10.7±0.5 BC
	14	9.6±0.6DE
	28	10.3±0.3 CD
	42	10.9±0.5 BC
	LIME	10.0±0.5 CDE

*Values are presented as means±SD (n=12). Means followed by the same letter are not significantly different. The letter grouping is based on LSMeans (p<0.05).
[1] ATB rates are all wet basis.

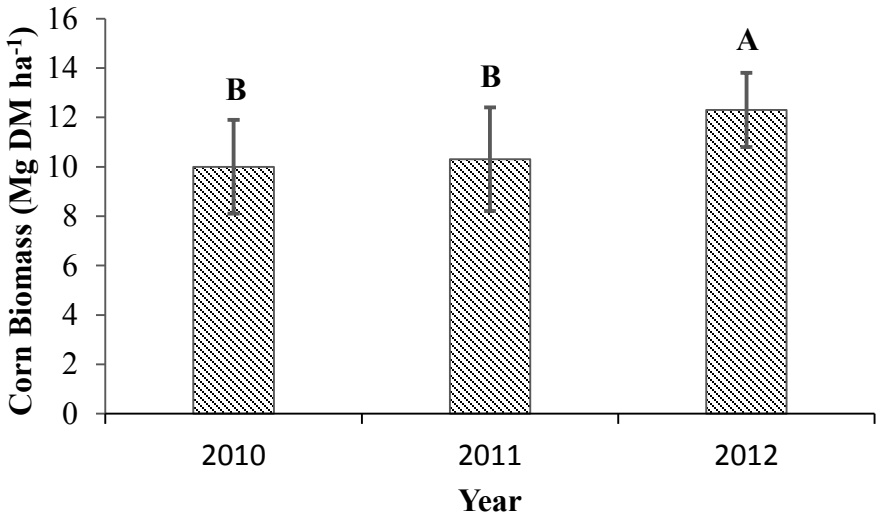

Figure 3.2 Corn biomass yield (Mg DM ha^{-1}) measured over both management practices and Alkaline Treated Biosolids (ATB) application rates in 2010, 2011 and 2012.

The ATB treatment rate had a significant effect on corn grain yield in 2009 (Table 3.5). No statistical differences were found among 7, 14 and 42 Mg ha^{-1} ATB for grain yield (Table 3.5). Applying 28 Mg ha^{-1} ATB resulted in no differences in grain yield from the two lower rate ATB applications and control. All of the ATB applications had higher yield than Lime. No differences were detected between lime treatment and control on grain yield (Table 3.5).

Table 3.5 Effect of Alkaline Treated Biosolids (ATB) application rates on corn grain yield (Mg DM ha^{-1}) in 2009.

ATB rates (Mg ha^{-1})[1]	Grain (Mg DM ha^{-1})
0	8.2±0.3 CD
7	9.4±0.2 AB
14	9.2±0.2 AB
28	8.9±0.3 BC
42	9.7±0.2 A
LIME	8.0±0.4 D

*Values are presented as means±SD (n=8). Means followed by the same letter are not significantly different. The letter grouping is based on LSMeans (p<0.05).
[1] ATB rates are all wet basis.

The corn grain yield was highly affected by the three way interaction of year x management practices x ATB rates (Table 3.6). For comparing the corn grain yield measured under each of the ATB rate across year, all the annual and single ATB application rates showed a trend of increasing over three years (Table 3.6). For the corn grain yield measured within each year, ATB rates effect was detected under annual ATB application for year 2010, 2011 and 2012 that higher ATB rates resulted in higher soil pH than low ATB rates application. Similar trend was detected under single ATB application in 2010 as well. When comparing the corn grain yield measured across the management practice, annual high rates of ATB application resulted in higher corn grain yield when compared to single corresponding rate of ATB application in year 2011 and 2012. However, this trend was not detected in year 2010 (Table 3.6).

Table 3.6 Effect of Year x Management practice x Alkaline Treated Biosolids (ATB) rates interactions on corn grain yield (Mg DM ha^{-1}) from 2010 to 2012.

Management practices	ATB rates (Mg ha^{-1})[1]	Year		
		2010	2011	2012
Annual	0	B 5.3±0.5 e	B 5.6±0.5 cd	A 8.1±0.8 c
	7	B 6.5±0.9 bcd	B 6.1±1.1 bcd	A 9.2±0.9 bc
	14	B 7.1±0.4 ab	B 8.2±1.0 a	A 9.7±0.6 ab
	28	B 7.9±1.5 a	B 9.0±0.2 a	A 10.8±0.5 a
	42	B 7.9±2.3 a	B 9.1±0.4 a	A 10.8±0.7 a
	LIME	B 5.9±0.6 cde	B 5.9±1.0 bcd	A 8.2±0.5 c
Single	0	B 5.4±0.8 de	B 6.0±0.8 bcd	A 8.2±0.5 c
	7	B 5.8±0.6 de	B 6.9±0.8 b	A 8.4±0.4 c
	14	B 6.1±1.0 bcde	B 5.3±0.7 d	A 8.9±0.6 bc
	28	B 7.0±0.4 abc	B 6.8±0.6 b	A 8.6±0.5 bc
	42	B 7.0±1.0 ab	B 6.3±0.9 bc	A 8.8±0.5 bc
	LIME	B 5.7±0.4 de	B 5.8±0.7 bcd	A 8.8±0.3 bc

*Values are presented as means±SD (n=4). Means followed by the same letter within each ATB rate over years (upper case) or within each year over ATB rates and management practice (lower case) are not significantly different (p<0.05). The letter grouping is based on LSMeans.

[1] ATB rates are all wet basis.

3.3.3 Plant P uptake

No significant ATB rates effect on BPU was detected for the year of 2009, which resulted in an average of 33.0±0.7 kg P ha^{-1} (n=48), while significant differences were detected in the 2009 GPU data. The GPU measured under 42 Mg ha^{-1} ATB was not significantly different from 7 and 14 Mg ha^{-1} ATB (Table 3.7). Plant uptake P for the ATB treatment of 28 Mg ha^{-1} was not significantly different from the other ATB rates except 42 Mg ha^{-1} ATB. Lime treatment showed no differences on BPU from control and all the ATB rates except the 42 Mg ha^{-1} ATB.

Table 3.7 Effect of Alkaline Treated Biosolids (ATB) application rates on corn grain P uptake (GPU) (kg P ha^{-1}) in 2009.

ATB (Mg ha^{-1})[1]	GPU (kg P ha^{-1})
0	24.3±1.1 C
7	28.7±0.5 AB
14	28.6±1.0 AB
28	25.8±1.3 BC
42	30.6±1.2 A
LIME	25.6±1.8 BC

*Values are presented as means±SD (n=8). Means followed by the same letter are not significantly different. The letter grouping is based on LSMeans (p<0.05).
[1] ATB rates are all wet basis.

The BPU measured under annual 14 and 28 Mg ha^{-1} ATB was significantly higher than single corresponding ATB rates (Table 3.8). The BPU measured under annual 28 Mg ha^{-1} ATB application was significantly higher than all ATB rates under both management practices except 42 Mg ha^{-1} ATB. The BPU measured under the annual 14 and both annual and single 42 Mg ha^{-1} ATB were not statistically different from each other but were significantly higher than all the other ATB rates (except annual 28 Mg ha^{-1} ATB). The BPU measured when adding 7 Mg ha^{-1} ATB annually showed no statistic differences from all

the single ATB and lime application treatments, but was significantly higher than control. Comparing the BPU measured within single ATB application, single 42 Mg ha^{-1}ATB was not different from 28 Mg ha^{-1} ATB but significantly higher than rest of the single ATB rates. No significant differences in BPU were detected among the single treatment of lime, 7, 14, and 28 Mg ha^{-1} ATB. The BPU measured under both annual and single ATB application were statistically higher than control (Table 3.8). The BPU measured in year 2010 and 2011 were not different from each other but lower than the year 2012 (Figure 3.3).

Table 3.8 Effect of Management practice x Alkaline Treated Biosolids (ATB) rates interactions on corn biomass P uptake (BPU) (kg P ha^{-1}) from 2010 to 2012.

Management practices	ATB (Mg ha^{-1})[1]	BPU (kg P ha-1)
Annual	0	19.5±0.9 F
	7	25.1±1.4 CD
	14	28.0±1.0 B
	28	31.2±1.0 A
	42	29.1±1.1 AB
	LIME	22.9±1.2 DE
Single	0	20.3±1.0 EF
	7	23.9±1.2 D
	14	22.9±1.4 D
	28	24.6±1.0 CD
	42	27.0±1.3 BC
	LIME	23.1±1.2 D

*Values are presented as means±SD (n=12). Means followed by the same letter are not significantly different. The letter grouping is based on LSMeans (p<0.05).
[1] ATB rates are all wet basis.

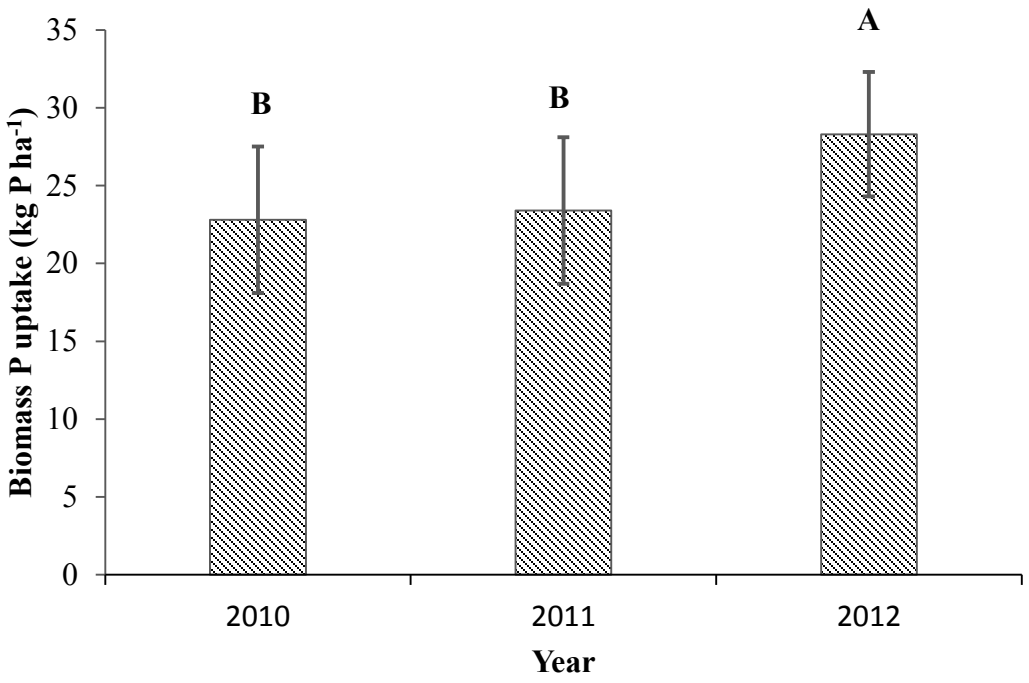

Figure 3.3 Corn biomass P uptake (BPU) (kg P ha^{-1}) measured over both management practices and Alkaline Treated Biosolids (ATB) application rates in 2010, 2011 and 2012.

The GPU concentrations measured under annual 14, 28 and 42 Mg ha^{-1} ATB were statistically higher than the corresponding single ATB application (Table 3.9). The GPU measured under annual ATB applications of 14, 28 and 42 Mg ha^{-1} were significantly higher than all the other ATB rates under both management practices, but they were not significantly higher than one another. Annual application of 7 Mg ha^{-1} ATB resulted in no differences on P uptake with all the single ATB and lime application but higher than control. For the single ATB application only, no significant differences on GPU were detected among all the ATB rates and control except 28 Mg ha^{-1} ATB, which was higher than control and 14 Mg ha^{-1} ATB. Adding lime one-time or annually was not different from control on GPU. All the annual ATB treatments were significantly higher than control (Table 3.9).

Table 3.9 Effect of Management practice x Alkaline Treated Biosolids (ATB) rates interactions on corn grain P uptake (GPU) (kg P ha^{-1}) from 2010 to 2012.

Management practices	ATB rates (Mg ha^{-1})[1]	GPU (kg P ha^{-1})
Annual	0	19.1±1.2 E
	7	23.9±1.7 BC
	14	27.3±1.4 A
	28	28.5±1.6 A
	42	29.1±1.6 A
	LIME	20.8±1.1 DE
Single	0	20.8±1.5DE
	7	22.7±1.3 BCD
	14	21.9±1.7 CD
	28	23.0±1.2 BCD
	42	24.2±1.4 B
	LIME	22.1±1.5 BCD

*Values are presented as means±SD (n=12). Means followed by the same letter are not significantly different. The letter grouping is based on LSMeans (p<0.05).
[1] ATB rates are all wet basis.

The effect of year x management practice on GPU from 2010 to 2012 is shown in Table 3.10. The GPU measured under annual ATB application across all the ATB rates showed an increasing trend over three years. Similar trend was detected under the single ATB application on GPU while no significant difference was detected between the GPU measured in year 2010 and 2011. Across the management practices, the GPU measured in each year under annual ATB application was not different from the single ATB application of the corresponding year except 2011 that GPU (Table 3.10).

Table 3.10 Effect of Year x Management practice interactions on corn grain P uptake (GPU) (kg P ha^{-1}) from 2010 to 2012.

Management practices	Year	GPU (kg P ha^{-1})
Annual	2010	21.0±4.7 C
	2011	23.1±5.4 B
	2012	29.9±4.6 A
Single	2010	19.7±2.9 CD
	2011	19.4±2.8 D
	2012	28.3±2.7 A

*Values are presented as means±SD (n=24). Different letters indicate the significant differences based on LSMeans (p<0.05).

In order to highlight the corn P uptake response over time, corn biomass and grain P uptake were expressed as percentages of the P uptake of ATB rate treatment over the control for the corresponding year, i.e. BPU and GPU ratio. The effect of year x management practice interactions on BPU ratio from 2010 to 2012 is shown in Table 3.10. The BPU ratio measured under annual ATB application across all the ATB rates showed a trend of decreasing over three years, while no specific trend was detected under the single ATB application. The BPU ratio measured within each year under annual ATB application was higher than the single ATB application except in 2012 (Table 3.11).

Table 3.11 Effect of Year x Management practice interactions on corn biomass P uptake (BPU) ratio from 2010 to 2012.

Management practices	Year	BPU ratio[1]
Annual	2010	1.49±0.31 A
	2011	1.45±0.23 A
	2012	1.31±0.20 B
Single	2010	1.26±0.24 B
	2011	1.13±0.19 C
	2012	1.24±0.16 BC

*Values are presented as means±SD (n=20). Different letters indicate the significant differences based on LSMeans ($p<0.05$).
[1] BPU = BPU ATB treatment/BPU unamended control.

The BPU ratio measured under annual 14, 28 and 42 Mg ha^{-1} ATB were statistically higher than the corresponding single ATB application (Table 3.12). The BPU ratio measured under either annual or single ATB applications showed a trend of increasing with the increasing of ATB rates (Table 3.12). The ATB rates effect on GPU ratio was detected under the annual ATB application, but this effect was not detected under the single ATB application (Table 3.13). Across the management practices, applying 14, 28, and 42 Mg ha^{-1} ATB annually resulted in higher GPU ratio than the corresponding one-time rate of ATB (Table 3.13). No significant relationship was detected between corn P uptake and M3P, as well as WEP in this study. However, a significant positive linear relationship was detected between corn P uptake and soil TP under both management practices with a slope of 0.031 (R^2=0.753, $p<0.05$) (Figure 3.4). A significant relationship was detected between corn P uptake and soil pH in the single ATB application, as well as with a slope of 14.34 (R^2=0.514, $p<0.05$) (Figure 3.5).

Table 3.12 Effect of Management practice x Alkaline Treated Biosolids (ATB) rates interactions on corn biomass P uptake (BPU) ratio from 2010 to 2012.

Management practices	ATB rates (Mg ha^{-1})[1]	BPU ratio[2]
Annual	7	1.30±0.19 DE
	14	1.45±0.19 BC
	28	1.63±0.23 A
	42	1.54±0.25 AB
	LIME	1.18±0.15 DE
Single	7	1.18±0.18 E
	14	1.13±0.20 E
	28	1.25±0.14 DE
	42	1.33±0.26 CD
	LIME	1.15±0.19 E

*Values are presented as means±SD (n=12). Different letters indicate the significant differences based on LSMeans (p<0.05).
[1] ATB rates are all wet basis.
[2] BPU ratio = BPU ATB treatment/BPU unamended control.

Table 3.13 Effect of Management practice x Alkaline Treated Biosolids (ATB) rates interactions on corn grain P uptake (GPU) ratio from 2010 to 2012.

Management practices	ATB rates (Mg ha^{-1})[1]	GPU ratio[2]
Annual	7	1.25±0.15 C
	14	1.42±0.18 B
	28	1.58±0.28 A
	42	1.61±0.25 A
	LIME	1.10±0.12 D
Single	7	1.12±0.23 CD
	14	1.06±0.18 CD
	28	1.15±0.23 CD
	42	1.17±0.24 CD
	LIME	1.09±0.25 CD

*Values are presented as means±SD (n=12). Different letters indicate the significant differences based on LSMeans (p<0.05).
[1] ATB rates are all wet basis.
[2] GPU ratio = GPU ATB treatment/GPU unamended control.

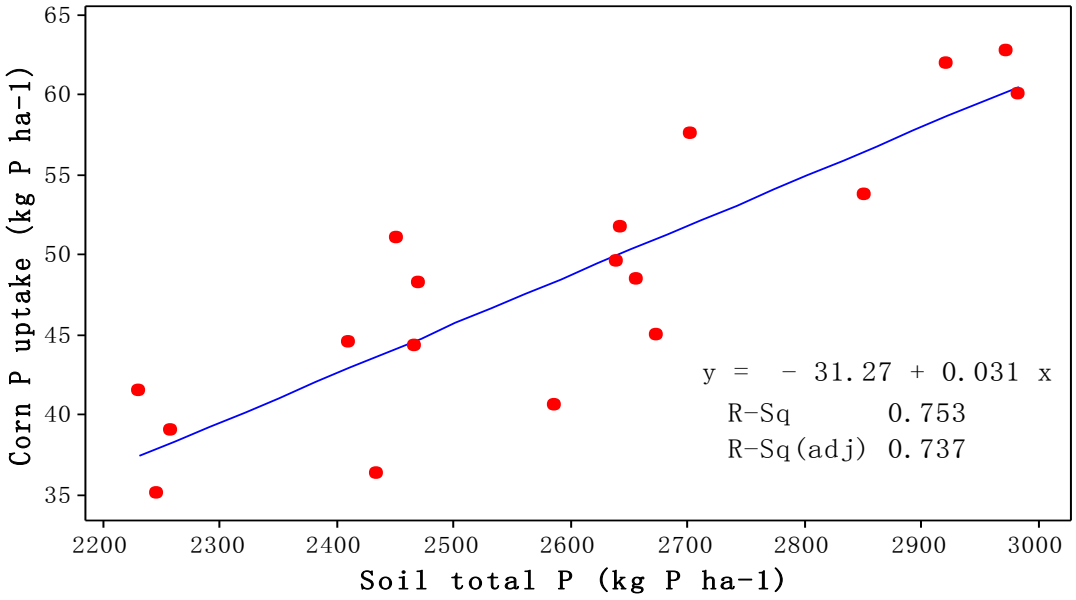

Figure 3.4 Relationship between soil total P (TP) (kg P ha^{-1}) and corn P uptake (kg P ha^{-1}) under both management practices from 2010 to 2012 (Corn P uptake=Biomass P uptake+ Grain P uptake).

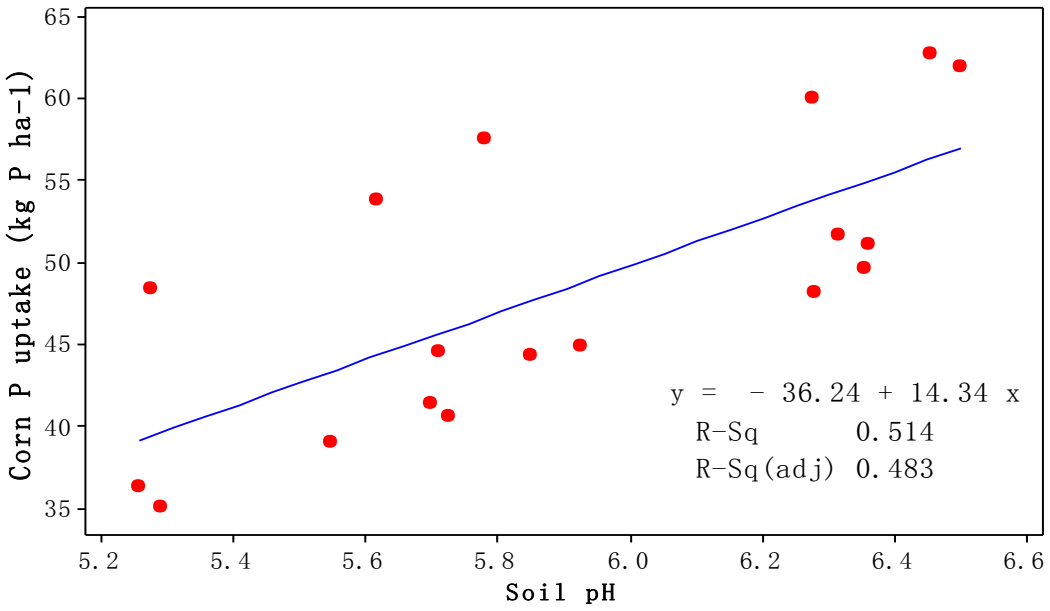

Figure 3.5 Relationship between soil pH and corn P uptake (kg P ha^{-1}) under both management practices from 2010 to 2012 (Corn P uptake=Biomass P uptake+ Grain P uptake).

3.4 Discussion

The slopes of 0.031 and 14.34 based on the significant relationship between crop P uptake and soil TP, as well as soil pH, suggest that an increase of 1 kg ha^{-1} total corn P uptake would require the increase of either 32.25 kg P ha^{-1} soil TP or 0.07 unit pH. These results indicate that increases in corn P uptake were mainly related to changes in soil pH and soil TP, rather than M3P and WEP. Both M3P and WEP may not be the good indicator of plant available P in this acidic soil with excessive P.

The corn grain yield data showed that the grain yield in the control and lime plots soared in 2012 regardless of the management practices (Table 3.6). A similar trend was observed in corn biomass yield, BPU and GPU across all the ATB rates and management practices in that same year (Figure 3.2, 3.3 and Table 3.10). The increase in crop yield and P uptake were not observed for the BPU and GPU ratios which indicates that these results are not due to the ATB application or residual P effect but for other reasons. Climatic condition is one possible limiting factor that can affect corn production. Markulj (2010) stated that a summer season with lower precipitation and higher temperature was less favorable for maize than a cooler summer with higher quantities of rainfall. In the present study, the average temperature over the summer period (June-August) in 2012 was 1.5°C higher than 2011, while the total precipitation during the summer season in 2012 was 36.0% drier and 2011 was 27.6% wetter than the long-term mean (1971-2000). This indicates that precipitation and temperature may not be the reason for the unexpected high uptake in the present study. In 2012, the inorganic fertilizer applied to all the plots was approximately double the amount of nitrogen (46.05 kg N ha^{-1}) than had been applied in previous years, which may explain the anomalous crop yield and P uptake observed in 2012. Marschner

(1995) reported that the N level increases resulted in P uptake increases due to a non-specific N-P interaction which was induced by the application of N fertilizer.

Residual P effect was observed based on the single ATB application result for crop yield and P uptake. After the one-time ATB application in 2009, the crop yield and P uptake measured under each ATB rate did not show a trend of decreasing even after three years and no ATB rates effect was detected within each year. The constant crop yield and P uptake under the single application is probably the result of high P mineralization at this site resulting in a residual plant available P pool (Nuruzzaman et al. 2005). Sattari et al. (2012) reported that corn P uptake kept rising in many European countries after the reduction of P fertilizer application rates since the 1980s. Considering the residual P accumulated in the soil can help to reduce the fertilizer use in the future. The consistent crop yield and P uptake under single ATB application was probably due to the slow release of plant available N from the added biosolids treatments (Cooper 2005). Although residual N and P in this soil can help to maintain the crop P uptake and crop yield, better crop responses still require the additional nutrient supply from ATB. The three year average crop P uptake and yield results show that annual high rates of ATB application resulted in better crop responses when compared to the other ATB rates under both management practices. This can be explained by the soil test results showed in Chapter two. The application of ATB at high rates annually increased the soil pH which decreased the P adsorption by reducing the activity of exchangeable Al and Fe and increased the plant available P in the soil. Since no relationship was observed between M3P and crop P uptake, as well as between WEP and crop P uptake, the higher corn yield and P uptake measured under the annual high ATB rates of application may be due to other non-P effect of ATB

or fertilizer management. The increase of soil organic matter (SOM) with the application of biosolids was reported by several studies (Tian et al. 2009; Wallace et al. 2009). This may be the case in this present study that annual ATB application increased the SOM when compared to the single ATB application which may help to explain the higher crop responses observed under the annual ATB application. The ATB application rate in this study was based on the corn N requirements instead of P. The total N applied over the study period from ATB was 270, 540 and 810 kg N ha^{-1} for the rate of 14, 28 and 42 Mg ha^{-1} ATB from 2010 to 2012, respectively. The high concentration of N applied from ATB may also help to explain the better crop responses under the annual high ATB application which also impacted the P dynamics in this system.

3.4 Conclusion

Overall, a positive effect of increasing ATB rates was observed on crop yield and P uptake under the annual ATB application. No ATB rates effect was detected under the single ATB application. However, a residual P effect was observed based on the single ATB application for the measured crop responses. When compared across the management practices, annual high rates of ATB application resulted in higher crop responses than the corresponding single ATB application rate.

4 Conclusion

This study shows that annual high ATB application rates increase soil P concentrations and crop P uptake and yield when compared with annual low rates and single ATB applications.

The plant available P measured by Mehlich 3 and water extractions were greater with higher ATB application rates. However, management practice did not result in significant differences in M3P measured in this study but annual high ATB application rates increased WEP over a single ATB application. Annual ATB application did not show a trend of M3P increasing from 2010 to 2012, which is contrary to our hypotheses and the M3P level was constant over time in one-time ATB application treatments due to the continuous soil available P provided by the soil residual P pool. No relationship was detected between WEP and M3P due to the narrow range of WEP concentrations measured in this study.

The soil TP measured in this study under both management practices for each of the ATB rate did not show a trend of increasing over time which may be due to the high variability in this site or the applied P from ATB not being retained in this soil. No management practices effect was observed on soil TP as well. The soil TP and M3P were well correlated suggesting soil TP might have potential to estimate M3P in the soil and is deserving of further study. Under annual ATB application, P accumulation could potentially occur particularly at the 28 and 42 Mg ha^{-1} ATB rates. These results partly prove our hypotheses that P accumulation will happen under the annual high rates of ATB application is likely.

The soil pH results in this study showed that ATB can be used as an alternative for agricultural lime but farmers will need to adjust the ATB applied to the soil in order to

achieve the same results. The optimum pH level was measured on the annual 28 and 42 Mg ha^{-1} ATB application and management practices had significant effects on increasing soil pH, especially under annual ATB application.

Both the M3Al and M3Fe showed an overall trend of decreasing with the ATB application rate increasing under annual ATB application. However, the management practices had no significant effect on the reduction of extractable Al and Fe in this study. The change of M3Al and M3Fe had an opposite trend to soil pH under both management practices because both M3Al and M3Fe were negatively correlated with soil pH. The increase in soil pH due to the ATB application help to avoid the Al toxicity, which decrease the M3Al and M3Fe level and increase the plant available P because the reduction of P sorption. This is further approved by the negative linear relationship between M3P and M3Al in the soil. Increased the amount of P applied from ATB will result in higher PSI based on the good correlation between these two response variables. In this study, most of the PSI measured fall into the category of "below optimum" except with the 28 and 42 Mg ha^{-1} ATB application, which increased the PSI to "above optimum". While these results suggest that applying high ATB rates annually would result in low P related environmental concerns in this site, concerns related to the effectiveness of using M3P as a part of the index were raised in this study. An increase in WEP under annual 28 and 42 Mg ha^{-1} ATB suggests a greater risk of P leaching and runoff if applied over an extended period of years.

For the effect of ATB application on crop responses, the ATB rates effect was detected under annual ATB application that higher rates of ATB application resulted in better crop responses over the four year study which could also be a result of additional N applied from ATB or fertilizer. Good linear relationships were detected between crop P

uptake and soil TP as well as soil pH which indicates that increases in crop P uptake were mainly related to changes in soil pH and soil TP, rather than M3P and WEP. When comparing the crop responses across the management practices, annual high rates of ATB application resulted in superior crop uptake and yield than single application. Residual effects were detected after four years following the single application of ATB in 2009.

Overall, the results of this study indicated that applying high rates of ATB annually increased the soil pH, plant available P and crop P uptake over four years The potential for soil P accumulation and elevated risk of P leaching and runoff needs to be considered when using the high rates of ATB annually for extended periods of time, particularly in soils with a history with a high total P.

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APPENDIX A. Nitric acid microwave digestion method

Air dried <2 mm soil was weighed 0.5g onto a Whatman #41 70mm Ø ashless filter paper and the soil weights were recorded to 4 decimals places. The filter paper was folded into a pouch and inserted into a clean dry reaction vessel using a glass stirring rod to push the sample to the bottom of the vessel. In a fume hood, 10.00 ml of concentrated nitric acid was added to each vessel using a bottle top dispenser. The samples were reacted with nitric acid for 15 minutes prior to placing the pressure discs on and tightening the caps. The capped vessels were fully inserted into the composite sleeves in the turntable with a minimum number of 8 vessels each run. The total P program in MARS xpress microwave digestor was specified as below:

Method Name:	TOTAL P 8 TO 20 (8 to 20 vessels)
Sample Type:	organic
Ramp time:	20 minutes
Temperature:	180 C
Hold time:	15 minutes
Power:	1600W
Percent Power:	60%

Method:	TOTAL P 21 TO 40 (21 to 40 vessels)
Sample Type:	organic
Ramp time:	20 minutes
Temperature:	180 C
Hold time:	15 minutes
Power:	1600W
Percent Power:	100%

A 30 minute cool down time was required to prevent dangerous boiling of acid after the digestion. When the run was completed fully, the turntable was removed from the MARS, from which individual vessels were removed and placed in a rack. A 100 ml volumetric flask with a funnel and a Fisherbrand Q8 125mm Ø filter paper was set up for each sample and each blank. After transferring all the contents of the vessel quantitatively to the

volumetric flask with deionized water, volume of liquid in the flasks was adjusted to 100ml. The diluted samples were transferred into Fisherbrand 50ml polypropylene centrifuge tubes and subsequently analyzed without further dilution by ICP-OES based on the AOAC method 985.01 (Horwitz and Latimer 2011) at the Nova Scotia Department of Agriculture Laboratory Services, Bible Hill, NS.

A dry run using reference soil was conducted to test the accuracy of measuring total P by the nitric acid microwave digestion method in soil samples. The reference soil (TILL-2) was collected and characterized in cooperation with the Mineral Resources Division, Minerals and Continental Geoscience Branch, Geological Survey of Canada. The reference soil used in this study was collected near Scission's Brook, New Brunswick.